# How to see the invisible?

# An objective approach to cognitive fatigue diagnosis and treatment evaluation in people with multiple sclerosis

## Thesis

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# List of abbreviations

| | |
|---|---|
| **ADHD** | Attention-Deficit / Hyperactivity Disorder |
| **AIC** | Akaike information criterion |
| **ANOVA** | Analysis of Variance |
| **AX-CPT** | AX continuous performance task |
| **BDI-II** | Beck Depressions-Inventory, 2. Version |
| **BDI-II-FS** | Beck Depressions-Inventory, Fast-Screen for Medical Patients |
| **BL** | Baseline |
| **CIS** | Clinical Isolated Syndrome |
| **CNS** | Central Nervous System |
| **DLPFC** | Dorso-Lateral Prefrontal Cortex |
| **DMT** | Disease-modifying therapy |
| **EDSS** | Expanded Disability Status Scale |
| **EEG** | Electroencephalography |
| **EMG** | Electromyography |
| **EOG** | Electrooculography |
| **ERP** | Event-Related Potential |
| **ESS** | Epworth Sleepiness Scale |
| **FIS** | Fatigue Impact Scale |
| **FM-Theta** | Frontomedial Theta |
| **FSMC** | Fatigue Scale for Motor and Cognitive Functions |
| **FSS** | Fatigue Severity Scale |
| **GLMM** | Generalized Linear Mixed Model |
| **HC** | Healthy Controls |
| **IAF** | Individual Alpha Frequency |
| **ISI** | Interstimulus-Interval |
| **L-COF** | Long-Covid-related Fatigue |

| | |
|---|---|
| **LMM** | Linear Mixed Model |
| **MFIS** | Modified Fatigue Impact Scale |
| **MRI** | Magnetic Resonance Imaging |
| **MS** | Multiple Sclerosis |
| **NIBS** | Non-Invasive Brain Stimulation |
| **NRS** | Numerical Rating Scale |
| **PASAT** | Paced Auditory Serial Addition Test |
| **PFS** | Pittsburgh Fatigability Scale |
| **PPC** | Posterior Parietal Cortex |
| **PPI** | Prepulse Inhibition |
| **PP-MS** | Primary Progressive MS |
| **PVT** | Psychomotor Vigilance Test |
| **pwMS** | People with Multiple Sclerosis |
| **ROPE** | Region of Practical Equivalence |
| **RR-MS** | Relapsing-Remitting MS |
| **RT** | Reaction Time |
| **SD** | Standard Deviation |
| **SDMT** | Symbol Digit Modalities Test |
| **SP-MS** | Secondary Progressive MS |
| **SRT** | Simple Reaction Time Task |
| **tACS** | Transcranial Alternating Current Stimulation |
| **TAP** | Test battery of Attentional Performance |
| **taVNS** | Transcutaneous Auricular Vagus Nerve Stimulation |
| **tDCS** | Transcranial Direct Current Stimulation |
| **tES** | Transcranial Electrical Stimulation |
| **VAS** | Visual Analog Scale |
| **WEIMuS** | Wuerzburg Exhaustion Inventory in Multiple Sclerosis |

# Abstract

Fatigue refers to a subjective lack of mental or physical exhaustion. It is a complex syndrome of many neurological diseases. It also affects up to 80% of people with multiple sclerosis (pwMS) and drastically limits their quality of life. However, despite its high social and clinical significance, progress in understanding and treating the syndrome is still limited. Part of this is due to the subjective definition that makes the syndrome diagnostically invisible. Besides subjective fatigue, however, there is also an objectively measurable decline in performance - fatigability. However, previous studies on objectively measurable parameters, particularly behavioral parameters, yielded controversial results and showed contradictory associations with subjective fatigue perception. This further complicates the evaluation of alternative treatments for fatigue and fatigability, such as transcranial direct current stimulation (tDCS). Therefore, this thesis aims (i) to complement the subjective fatigue diagnosis with objective electrophysiological parameters and (ii) to investigate the effects of frontal tDCS on subjective fatigue and objectively measurable fatigability in both healthy subjects as well as pwMS.

This thesis includes one review article (Project A) and four empirical studies (Project B and C). In Project A, I presented a comprehensive overview of the current literature and developed a unified fatigue taxonomy. Furthermore, I elaborated on the relevance of distinguishing between MS-related fatigue and fatigability and discussed the lack of a correlation between current objective parameters and subjective fatigue. In Project B, I examined the effects of repetitive tDCS on fatigue and fatigability symptoms in pwMS. Subjective fatigue improved; however, it did so equally in the verum as well as in the placebo condition. In contrast, the stimulations did not affect fatigability, as measured by changes in reaction time and P300 amplitudes. Project C included three empirical studies (Project C1-C3) in which I focused on four potential electrophysiological fatigue and fatigability parameters: frontomedial theta power, occipital alpha power, prepulse inhibition (PPI), and P50 sensory gating. Project C1 examined these parameters in young, healthy subjects and additionally explored how they are affected by frontal tDCS. In Projects C2 and C3, I subsequently examined the parameters in pwMS and age-matched controls. In summary, the results of these three

empirical studies showed a fatigability-related increase in occipital alpha power as well as a decline in the gating ratios. A single tDCS session counteracted fatigability development and resulted in lower fluctuations. In pwMS, I additionally demonstrated a lack of a theta power increase, as well as a relationship between P50 sensory gating and subjective fatigue scores.

In conclusion, this thesis provides important results that expand the understanding of MS-related fatigue and fatigability. I present several objective and reliable electrophysiological parameters that can complement the purely subjective fatigue diagnosis and can help to evaluate the effectiveness of frontal tDCS as an alternative fatigue treatment. Furthermore, the results presented in this thesis provide an essential foundation for future research.

# German Abstract (Zusammenfassung)

Fatigue wird als ein subjektiver Mangel an geistiger oder körperlicher Erschöpfung definiert. Es ist ein vielschichtiges Syndrom vieler neurologischer Erkrankungen. So betrifft es auch bis zu 80 % der Menschen mit Multipler Sklerose (MS) und schränkt ihre Lebensqualität drastisch ein. Trotz der großen sozialen und klinischen Bedeutung sind die Fortschritte beim Verständnis und bei der Behandlung des Syndroms jedoch noch begrenzt. Dies liegt zum Teil in der subjektiven Definition begründet, durch die das Syndrom diagnostisch unsichtbar ist. Neben der subjektiven Fatigue gibt es aber auch einen objektiv messbaren Leistungsabfall – die Fatigability. Bisherige Studien zu objektiv messbaren Parametern, insbesondere Verhaltensparametern, ergaben jedoch kontroverse Ergebnisse und zeigten widersprüchliche Zusammenhänge mit dem subjektiven Fatigue-Empfinden. Dies erschwert auch die Evaluation der transkraniellen Gleichstromstimulation (tDCS) als mögliche alternative Behandlung der MS-bedingten Fatigue und Fatigability. Die Ziele dieser Dissertation waren es daher, (i) die subjektive Fatigue-Diagnose durch objektive elektrophysiologische Parameter zu ergänzen und (ii) die Auswirkungen frontaler tDCS auf die subjektive Fatigue und die objektiv messbare Fatigability bei gesunden Menschen als auch bei Menschen mit MS zu untersuchen.

Diese Arbeit umfasst einen Übersichtsartikel (Projekt A) und vier empirische Studien (Projekt B und C). In Projekt A gab ich einen umfassenden Überblick über die aktuelle Literatur und entwickelte eine einheitliche Fatigue-Taxonomie. Darüber hinaus arbeitete ich die Relevanz heraus, zwischen MS-bedingter Fatigue und Fatigability zu unterscheiden und diskutierte die oft fehlende Korrelation zwischen aktuellen objektiven Parametern und subjektiver Fatigue. In Projekt B untersuchte ich die Auswirkungen repetitiver tDCS-Stimulationen auf die Fatigue- und Fatigability-Symptomatik bei Menschen mit MS. Die subjektive Fatigue verbesserte sich; tat dies jedoch sowohl in der Stimulations- als auch in der Placebobedingung. Keinen Einfluss hatten die Stimulationen dahingegen auf die Fatigability, die ich anhand von Reaktionszeitenveränderungen und P300 Amplitudenreduktion operationalisierte. Projekt C umfasste drei empirische Studien (Projekt C1-C3), in denen ich mich auf vier potenzielle elektrophysiologische Parameter konzentrierte: frontomediale Theta-Power, okzipitale Alpha-Power, Präpulsinhibition (PPI) und P50- Sensorisches Gating. Projekt C1 untersuchte diese Parameter bei jungen,

gesunden Menschen und untersuchte zusätzlich, wie die Paramter durch frontale tDCS-Stimulation beeinflusst werden. In den Projekten C2 und C3 untersuchte ich anschließend die Parameter bei Menschen mit MS und alters-gematchten Kontrollpersonen. Zusammenfassend zeigten die Ergebnisse dieser drei empirischen Studien einen ermüdungsbedingten Anstieg der okzipitalen Alpha-Power sowie eine Abnahme der Gating-Indizes. Eine einzige tDCS-Sitzung wirkte der Fatigability-Entwicklung entgegen und führte zu geringeren Schwankungen. Bei Menschen mit MS konnte ich darüber hinaus einen fehlenden Theta-Anstieg sowie einen Zusammenhang zwischen P50 sensorischem Gating und subjektiven Fatigue-Werten nachweisen.

Zusammengefasst liefert diese Dissertation wichtige Ergebnisse, die das Verständnis von MS-bedingter Fatigue und Fatigability erweitern. Ich stelle mehrere objektive und zuverlässige elektrophysiologische Parameter vor, die die rein subjektive Fatigue-Diagnose ergänzen und dazu beitragen können, die Wirksamkeit von frontalen tDCS-Stimulationen als alternative Fatigue-Behandlung besser zu bewerten. Darüber hinaus bilden die in dieser Arbeit vorgestellten Ergebnisse wichtige Grundlagen für künftige Forschungen.

# 1 General Introduction

## 1.1 Introducing the topic

This thesis focuses on the fatigue syndrome, a rather intangible and invisible syndrome. Those suffering from fatigue often describe it as a leaden tiredness that significantly limits daily activities and worsens over the course of one day. It can affect people with many neurological diseases. Especially among people with multiple sclerosis (pwMS), it represents one of the most common and worst syndromes. Hence, it affects up to 80 % of pwMS (Oliva Ramirez et al., 2021). Along with motor symptoms, it is considered to be the MS symptom that most severely reduces the quality of life in pwMS and leads to early retirement (Oliva Ramirez et al., 2021; Simmons et al., 2010; Yamout et al., 2013).

According to Chalah and Ayache (2018b), the fatigue syndrome includes symptoms such as increased exhaustion and tiredness with disturbed sleep patterns, difficulty concentrating, lack of motivation to perform daily activities, and feelings of generalized weakness. Subsequently, it may lead to motoric, cognitive, or psychosocial impairments (Fisk et al., 1994). However, even after many years of research, the pathophysiology and effective treatment options are still unknown. This is primarily due to the invisibility and subjective nature of fatigue, which complicates the assessment and quantification of fatigue. However, in order to develop effective treatment options, this would be a crucial first step.

In the following chapter, I will briefly introduce MS as the underlying neurological disease as this will help to understand the development and maintenance of fatigue in MS. I will then focus on fatigue and its pathophysiology as well as current diagnostics and treatment options. Finally, I will present the objectives of this thesis.

## 1.2 Multiple Sclerosis

Multiple sclerosis (MS) or encephalomyelitis disseminata is an immune-mediated encephalopathy causing inflammation in the central nervous system. It predominantly affects the white matter of the central nervous system (CNS), leading to demyelination and, subsequently, atrophy of neurons (*Pschyrembel*, 2020). MS affects approximately 2.3 million people worldwide and has a prevalence of 50 - 300 per 100 000 people (Thompson, Baranzini, et al., 2018). It is typically diagnosed between the ages of 20 and 40, and women are more likely to develop MS, with a roughly two- to threefold increased risk of developing the disease (Blaschke et al., 2022; Walton et al., 2020).

Interestingly, the prevalence of MS increases with increasing distance from the equator (McGinley et al., 2021; Thompson, Baranzini, et al., 2018). In higher latitudes, sunlight exposure is greater, and Vitamin-D concentrations are higher. Vitamin-D has a range of effects that also include immunoregulatory effects. As a result, both factors, sunlight exposure and Vitamin-D blood concentration, have been associated with a lower prevalence of MS (Lucas et al., 2011). Genetic factors may also contribute to MS, given the increased prevalence within families (McGinley et al., 2021). HLA, the leukocyte antigen located on the short arm of the 6th chromosome, has been the best-studied genetic link to date. Variations in this region lead to an increased or decreased risk of MS, depending on the type of variation. The HLA-DR1*15:01 carrier is the most frequent genetic factor associated with MS, conferring a threefold increase in the possibility of developing MS (Kamm et al., 2014; McGinley et al., 2021). Importantly, one recent study highlighted the crucial role of the Epstein-Barr virus in the chain of events that result in MS (Bjornevik et al., 2022). Thus, compared to controls, people who developed MS had a much higher rate of Epstein-Barr virus infection. Infected people were 32 times more likely to develop MS than non-infected people. However, the Epstein-Barr virus alone was insufficient to trigger MS. Other unknown factors certainly contribute to its development (Bjornevik et al., 2022).

### 1.2.1 Pathogenesis

Wildemann and Diem (2016) describe the pathogenesis of MS as follows: for reasons currently unclear, peripheral, autoreactive T- and B- lymphocytes are activated. By

mistake, these cells adhere to receptors and pass through the blood-brain barrier into the brain. They then recognize endogenous CNS structures as foreign antigens resulting in the release of proinflammatory cytokines. Among the structures falsely recognized as foreign is the myelin protein of the myelin sheath. As a result of the cytokine activation, macrophages, specifically B- cells, are activated. The interaction of T- and B- cells triggers an inflammatory cascade that leads to the degradation of oligodendrocytes, the cells that form the myelin sheath. The result is damage to the myelin sheath, leading to impaired or even completely absent nerve conduction. Depending on the focus of inflammation, this, in turn, leads to the typical MS symptoms. When the acute inflammation has subsided, MS glial scars (sclerosis) develop as a consequence of demyelination.

### 1.2.2 Classification and Symptoms

The MS types are classified according to their course of progression (see Figure 1). A distinction is made between relapsing-remitting MS (RR-MS), secondary progressive (SP-MS) and primary progressive MS (PP-MS; Lublin et al., 2014; Lublin & Reingold, 1996).

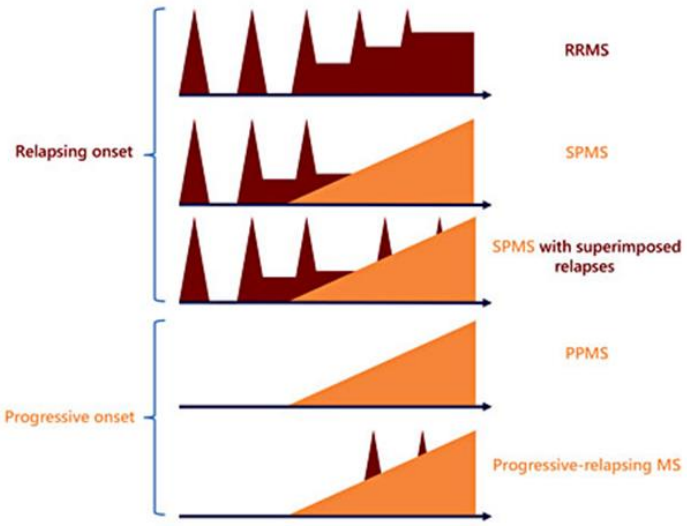


**Figure 1.** Clinical courses of MS (taken from Kamm et al., 2014)

RR-MS is the most common MS form, with a probability of 80% (Kamm et al., 2014; Wildemann & Diem, 2016). Clearly distinguishable relapses characterize it. A relapse is the occurrence or worsening of neurological symptoms without a clear cause. The symptoms must last at least 24 hours and be separated from the last relapse by at least one month (Wildemann & Diem, 2016). SP-MS, on the other hand, is characterized by the fact that it initially similarly begins with a relapsing course. Subsequently, however, there is a progressive worsening of the symptoms with permanent residual symptoms. In most pwMS with RR-MS (up to 75 %), the course of the disease later changes to SP-MS. PP-MS, on the other hand, is the rarest form, with 10-15%. In this case, there is a chronic progressive course from the beginning, in which mainly the motor symptoms continuously worsen (Kamm et al., 2014; Wildemann & Diem, 2016). In recent years, the clinically isolated syndrome (CIS) has also been included among the forms of MS. CIS refers to a clinical event that is highly suggestive of a demyelinating disease but has not yet manifested enough to diagnose MS. In most cases, the presenting symptoms are monofocal, evolve over a short period of time, and involve the optic nerve, spinal cord, brain stem, or cerebellum (Klineova & Lublin, 2018).

The symptomatology of MS is subject to substantial interindividual variability. The classic manifestations of MS include unilateral optic neuritis (blurred vision with accompanying pain), sensory disturbances (paresthesia) and numbness of the extremities, muscle weakness or dizziness and hearing loss (McGinley et al., 2021). Bladder and bowel dysfunction, as well as sexual dysfunction, are also among the common symptoms of MS. Additionally, cognitive and psychological symptoms, such as memory and attention deficits, as well as impaired cognitive flexibility may occur (Wildemann & Diem, 2016). A very large percentage of pwMS additionally suffer from fatigue, as already mentioned at the beginning of this thesis (Oliva Ramirez et al., 2021).

### 1.2.3 Diagnosis

As there is no specific laboratory parameter or diagnostic test for MS, the diagnosis of MS is primarily based on its clinical presentation. It is based on the McDonald criteria first described in 2001 (McDonald et al., 2001) and then revised in 2018 (Thompson, Banwell, et al., 2018). According to the criteria, an MS diagnosis is based on a combination of clinical examination, the exclusion of all differential diagnoses, magnetic

resonance imaging (MRI) and cerebrospinal fluid diagnosis. To diagnose MS, there must be at least two distinct onsets of symptoms, and they must be caused by at least two different inflammatory sites in the CNS (temporal and spatial dissemination). MRI can be used to detect those lesions in the CNS. Furthermore, a positive cerebrospinal fluid finding with oligoclonal bands and protein elevation may be present. Since the introduction of the McDonald criteria, a diagnosis of MS can be made even from the onset of the first episode (McGinley et al., 2021). The diagnosis is then classified as "definite MS", "possible MS", or "no MS" (Thompson, Banwell, et al., 2018).

### 1.2.4 Treatment

Despite years of research, MS has not yet been proven to be curable (Stangel et al., 2018). However, there are numerous treatment options, which differ depending on the progression status of MS. Thus, MS therapy includes acute relapse therapy, disease-modifying therapy, comorbidity management, psychological support and more (McGinley et al., 2021). During an acute relapse, the short-term use of high-dose, anti-inflammatory cortisone is used to achieve a remission of the acute clinical symptoms (Stangel et al., 2018). Disease-modifying therapy, on the other hand, is given to reduce the frequency of relapses (McGinley et al., 2021). Relapse activity determines which medication should be used. The following drugs are frequently used for mild relapses: Glatiramer acetate, Interferon-beta, Dimethyl fumarate, and Teriflunomide. In highly active MS courses, Cladribine, Fingolimod, Natalizumab, and Ocrelizumab are used. Ocrelizumab is also the only drug available for primary progressive forms of MS (Stangel et al., 2018).

## 1.3 Fatigue in people with multiple sclerosis

Fatigue is the most disabling and quality of life reducing symptom of MS. As already mentioned, unfortunately, about 80% of MS patients suffer from fatigue (Oliva Ramirez et al., 2021). The MS Council defines fatigue as follows:

> *"A subjective lack of physical and/or mental energy that is perceived by the individual or caregiver to interfere with usual and desired activities" (Multiple Sclerosis Council for Clinical Practice Guidelines, 1998, p. 2)*

This definition also illustrates the great challenge fatigue research faces. Thus, the general concept of fatigue is that it is a purely subjective, invisible syndrome. It is defined as a feeling "that is perceived", which leaves much room for interpretations of how each individual defines fatigue. As a result, reliable and valid fatigue measurements are difficult to assess, which is essential for progress in research. Kluger et al. (2013) also introduced an objective definition of fatigue, also referred to as fatigability. Thus, they define fatigability as objectively measurable changes in physical or cognitive performance relative to a reference value. According to the authors, fatigue and fatigability are not only distinct but also potentially independent constructs (Kluger et al., 2013). In addition, Genova et al. (2013) distinguish between trait and state fatigue. Thus, trait fatigue refers to the global status of an individual and changes only slowly over time. State fatigue, on the other hand, refers to the experienced fatigue during the performance of an exhaustive task. Thus, the current conceptualization of fatigue is based on two components:

*Fatigue* that is perceived and assessable via self-report questionnaires (trait) or rating scales (state)

*Fatigability* that is experienced with time on a physical or cognitive exhausting task and assessable via the change in an outcome variable (state)

## 1.3.1 Distinguishing fatigue from other constructs

### 1.3.1.1 Exhaustion and tiredness

The subjective perception of fatigue, as well as a performance decline during an exhaustive motor or cognitive task, are normal physiological reactions that any healthy individual can experience. However, in healthy subjects, those feelings are predictable and transient. Likewise, extensive tiredness in healthy subjects can almost always be traced back to inadequate sleep duration or quality. Clinically significant fatigue, in contrast, evolves much faster, does not resolve after sleep, is chronic, and is not predictable. The symptoms vary in their response to exertion or rest (Kluger et al., 2013; Multiple Sclerosis Council for Clinical Practice Guidelines, 1998). Furthermore, clinical

fatigue significantly decreases the quality of life and causes long-term disability (Simmons et al., 2010; Yamout et al., 2013).

Finally, it is essential to note that a lacking distinction may also be a matter of linguistics. Thus, in German, the term fatigue is almost exclusively used in a clinical context. In English, on the other hand, the terms “exhaustion”, “tiredness”, “sleepiness”, or “fatigue” are often interchangeably used by both clinical physicians as well as patients making it more difficult to distinguish between them.

#### 1.3.1.2 Depression

Fatigue and depression share a lot of common symptoms and, thus, often correlate (Kos et al., 2008; Sparasci et al., 2022). However, according to current knowledge, both constructs are considered independent constructs that may overlap clinically. In this respect, antidepressants are reported to be ineffective in relieving fatigue, in some cases even worsening it (Kuppuswamy, 2022). Fatigue may be a symptom of depression, but likewise, depressive symptoms may be the result of chronic fatigue and decreased life quality. Simply put, people with fatigue desire to be more active but are physically and mentally incapable, while people with depression lack the desire to be active.

#### 1.3.1.3 Cognitive impairment

Particularly when it comes to cognitive fatigue and fatigability, cognitive impairment is a reasonable possibility. In addition, many pwMS report cognitive dysfunctions (Bol et al., 2009). However, Hanken, Eling, and Hildebrandt (2015) reviewed the literature and reported no association between fatigue and memory performance, cognitive speed/ selective attention, and language or visuospatial processing in the majority of studies. A weak relationship was found between fatigue and working memory. In contrast, the authors reported a strong association between fatigue and alertness/ vigilance. In conclusion, the results of the review indicate that pwMS suffering from fatigue or fatigability and healthy controls show no difference in initial cognitive parameters (except alertness), but rather the differences become evident when sustained attention declines. Thus, according to Hanken, Eling, and Hildebrandt (2015), the cognitive process is not impaired as such, but fatigue distracts attention away from it, resulting in impairment.

### 1.3.2 Pathogenesis

Even after years of research, the exact cause of MS-related fatigue remains unclear. However, a variety of studies have described partly consistent and partly contradictory results. The pathogenesis of MS-related fatigue can be divided into primary and secondary fatigue (Ayache & Chalah, 2017; Kos et al., 2008). Primary fatigue refers to fatigue caused by the MS disease itself. Thus, one of the consequences of MS is the demyelination of neurons in the CNS. This subsequently leads to neuronal degeneration and/or cortical reorganization, which will have different effects on the complex processes in the CNS and consequently may lead to fatigue. In addition, fatigue may result from MS-related neuroimmune dysregulations due to increased levels of inflammatory mediators or neuroendocrine dysfunctions. Studies investigating primary fatigue will be further discussed below. In contrast, fatigue can also be a consequence of other MS-related comorbidities, such as sleep disturbance, depression, drug side effects, and more. Then it is called secondary fatigue. See Figure 2 for a schematic illustration of primary and secondary fatigue in MS.

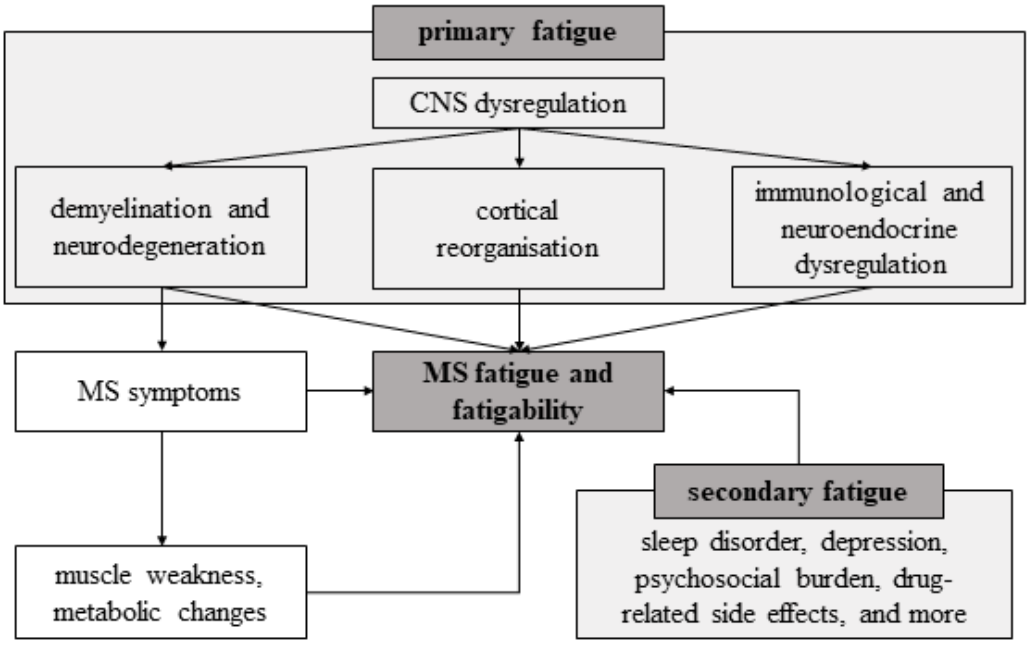


**Figure 2.** Schematic illustration of the pathogenesis of MS. Adapted from Ayache and Chalah (2017). *CNS,* central nervous system; *MS,* multiple sclerosis

The relationship between fatigue and lesion load has been reported in some studies. Thus, in high-fatigued pwMS, higher lesion load, as well as white and grey matter atrophy were demonstrated (Calabrese et al., 2010; Colombo et al., 2000; Cruz Gómez et al., 2013; Gonzalez Campo et al., 2020; Sander et al., 2017; Sepulcre et al., 2009; Tedeschi et al., 2007). Notably, an association between parietal lesions or cortical thickness and fatigue has repeatedly been reported (Colombo et al., 2000; Hanken, Eling, et al., 2016; Pellicano et al., 2010; Sepulcre et al., 2009). However, there is also controversial literature that reports no relationship between (global) lesion load and fatigue (Bakshi et al., 1999; Bisecco et al., 2018; Gobbi et al., 2014; Nourbakhsh et al., 2016; Palotai & Guttmann, 2020; Papadopoulou et al., 2013; Pravatà et al., 2016; Wilting et al., 2016).

Moreover, several studies on the relationship between fatigue and MS-related functional activity changes exist. Hence, demyelinated neurons and neurodegeneration may lead to underactivity in the respective areas. Therefore, to maintain functional capacity, it may be necessary to recruit more areas which would result in increased activity in other areas (Kos et al., 2008; Manjaly et al., 2019). This hypothesis has been confirmed by fMRI studies showing that pwMS with fatigue show an increase in distributed brain activity (Filippi et al., 2002; Leocani et al., 2001; Niepel et al., 2006; Roelcke et al., 1997). Filippi et al. (2002) found significant differences in activity between pwMS with and without fatigue in cortical and subcortical areas involved in motor planning and execution. Furthermore, many studies have found a relationship between fatigue and abnormal thalamic activity (Barbi et al., 2022; Bernitsas et al., 2017; Capone et al., 2020; Filippi et al., 2002; Inglese et al., 2007; Niepel et al., 2006; Wilting et al., 2016). Already in 1997, Roelcke et al. (1997) demonstrated reduced glucose metabolism in the lateral and medial prefrontal cortex, premotor cortex, as well as the putamen and caudate nucleus in pwMS with fatigue compared to pwMS without fatigue. Consistent with these findings, many functional studies have found activity or connectivity changes within frontal areas (Bisecco et al., 2018; Huolman et al., 2011; Pardini et al., 2010; Pravatà et al., 2016; Specogna et al., 2012) or between the basal ganglia and frontal areas (Chaudhuri & Behan, 2000; Derache et al., 2013; Finke et al., 2015; Inglese et al., 2007; Rocca et al., 2014; Téllez et al., 2008). Structural and functional abnormalities have also been found in sensory and motor areas (Tartaglia et al., 2004), insula, and anterior cingulate cortex (Gonzalez Campo et al., 2020), hypothalamus (Hanken, Eling, Kastrup,

et al., 2015; Hanken, Manousi, et al., 2016) and corpus callosum (Gobbi et al., 2014; Yaldizli et al., 2011; Yaldizli et al., 2014).

Furthermore, a few studies investigated brain activity changes during the performance of an exhausting task. The first study by DeLuca et al. (2008) investigated brain activity changes of pwMS while performing an exhaustive task. During an hour-long scanning session, subjects were required to perform a simple cognitive task repeatedly. The task was divided into four blocks, in which the first half was compared with the second half (within-runs). In addition, the authors examined general changes throughout the experiment (across-runs). Significant changes were found in frontal and parietal areas, occipital areas (within-runs), parietal areas and basal ganglia (across-runs). Another study particularly examined the relationship between brain activity of pwMS while performing a working memory task with self-reported fatigue (Engstroem et al., 2013). The authors found a positive correlation between state fatigue scores (assessed via VAS scores) and brain activation in the left posterior parietal cortex and the right substantia nigra, indicating that pwMS with higher state fatigue have higher activation in those areas. Finally, Genova et al. (2013) used diffusion tensor imaging and reported that subjective state fatigue was associated with hyperactivation of the caudate nucleus in pwMS. Increased subjective trait fatigue, on the other hand, was associated with a reduced fractional anisotropy in the anterior internal capsule.

In summary, these studies demonstrate an association between MS-related fatigue and fatigability and brain activity changes in the frontal brain, basal ganglia, striatum, and thalamus. Together, they provide evidence for a malfunctioning cortico-striato-thalamo-cortical network, the so-called fatigue circuit, underlying MS-fatigue (Ayache & Chalah, 2017; Chaudhuri & Behan, 2000). It remains unclear, however, whether and how the results are causally related.

Evidence for the immunological hypothesis comes from studies reporting increased tumor necrosis factor-α mRNA expression in peripheral blood cells of fatigued pwMS compared to non-fatigued pwMS, as well as a positive association between higher serum levels of interferon-γ levels and daytime sleepiness and fatigue severity (Flachenecker et al., 2004; Heesen et al., 2006). A review by Hanken et al. (2014) tried to integrate fatigue and fatigability by combining results from the neurodegenerative and the neuroinflammatory hypothesis into a coherent scheme (see Figure 3). They propose

that subjective trait fatigue results from peripherally released pro-inflammatory cytokines that activate immune-to-brain communication pathways. Those alter neural processing within interoceptive and homeostatic brain areas, distract endogenous attention, and disturb information processing. Vigilance decrements, on the other hand, may be a result of the previously described malfunctioning cortico-striato-thalamo-cortical network that leads to performance decline. Subjective trait fatigue may further exaggerate these alertness decrements by causing interoceptive interference.

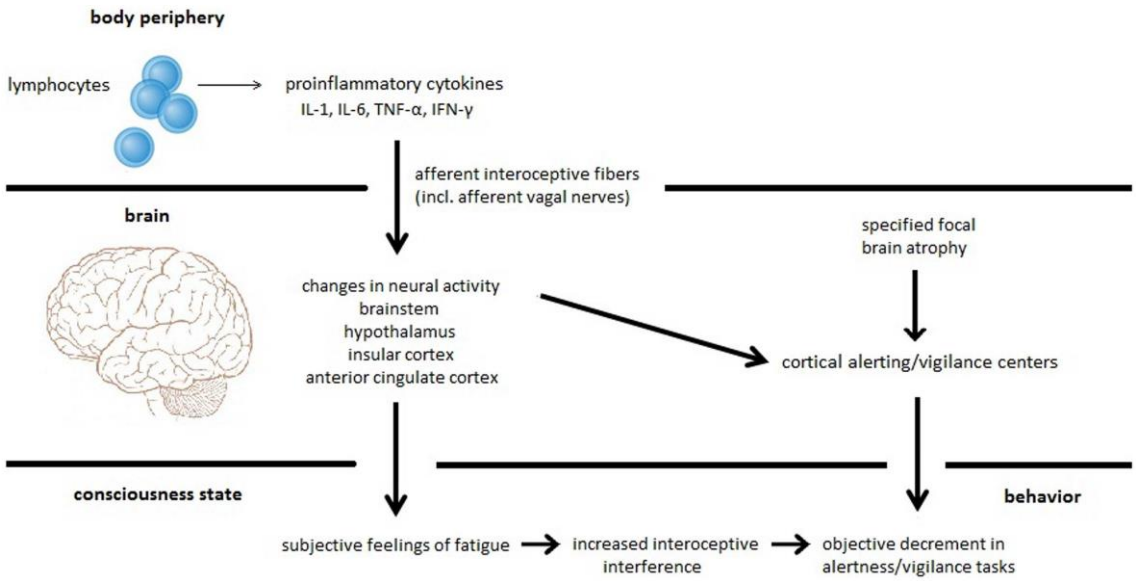


**Figure 3.** Proposed model for MS-related fatigue. Taken from Hanken et al. (2014).

### 1.3.3 Diagnosis

To date, fatigue is almost exclusively diagnosed based on subjective perception. There are about 250 scales to measure fatigue symptoms, of which 71 were created exclusively for fatigue diagnosis (Hjollund et al., 2007). The most commonly used questionnaires in MS-related fatigue are the Fatigue Severity Scale (FSS; Krupp et al., 1989), the Fatigue Impact Scale (FIS; Fisk et al., 1994), and the Modified Fatigue Impact Scale (MFIS; Multiple Sclerosis Council for Clinical Practice Guidelines, 1998). The FSS consists of nine items regarding fatigue severity, frequency, and impact on daily living. However, it

only exclusively considers the physical symptoms of fatigue. The FIS and MFIS, on the other hand, measure three dimensions of fatigue: the cognitive, physical, and psychosocial impairments caused by fatigue. The Fatigue Scale for Motoric and Cognition (FSMC; Penner et al., 2009) and the Wuerzburg Fatigue Inventory for Multiple Sclerosis (WEIMuS; Flachenecker et al., 2006) were explicitly developed for the German-speaking region. They measure the cognitive and physical dimensions, respectively.

The questionnaires used to assess fatigue have been criticized. Thus, they are subject to psychological biases. They retrospectively assess feelings of the last two to four weeks, therefore being susceptible to mood changes. Moreover, the questionnaires have only a slight to moderate correlation with one another, suggesting that they measure different aspects of the fatigue syndrome (Flachenecker et al., 2002).

In recent years, there has been growing interest in complementing the purely subjective diagnosis with objective parameters. Nevertheless, this has proven to be a very challenging task. This thesis will present several objective parameters for measuring fatigue and fatigability in pwMS, so this chapter only briefly introduces the topic. However, research on objective fatigue parameters is hindered by the fact that they rarely relate to subjective fatigue feelings (DeLuca, 2005). In this manner, although pwMS are frequently subjectively exhausted to an excessive degree, this may rarely be proven objectively.

### 1.3.4 Treatment

There is currently no uniform and effective fatigue treatment available. An essential component of the diagnostic process is the comprehensive history of the patient and the exclusion of possible differential diagnoses. These include the exclusion of sleep problems, drug-induced side effects, depression, or psychosocial stress. Following the exclusion of these factors, fatigue therapy can be categorized into medication-based and non-drug-based approaches.

Only two off-label prescribed drugs are currently available for treating MS-related fatigue. Amantadine is a virus-fighting agent commonly used in Parkinson's disease, and modafinil is a wakefulness-promoting agent (Veauthier & Paul, 2016). While

Amantadine rarely improves fatigue symptoms sufficiently, Modafinil has been shown to reduce fatigue in several studies (P. Miller & Soundy, 2017).

Non-pharmacological therapy can be divided into the following areas: education, lifestyle modification, energy conservation, or environmental modification (Khan et al., 2014). Exercise, in particular, has been shown to improve fatigue symptoms. For example, endurance training led to an improvement in 51% of the studies included in the meta-analysis, while strength training brought about an improvement in 68% of the studies (P. Miller & Soundy, 2017). Energy-saving strategies (Hersche et al., 2019; Mathiowetz et al., 2007) and cognitive-behavioral therapy (Chalah & Ayache, 2018a; van den Akker et al., 2017) also show promising short-terms improvements. For example, patients are advised to keep a fatigue diary to better understand cause and effect, to take several small breaks throughout the day, and to schedule important activities in the first half of the day. In a recent meta-analysis, balance training, as well as cognitive-behavioral therapy had the largest effect on fatigue compared to "treatment as usual" (Harrison et al., 2021). For fatigued pwMS whose symptoms worsen significantly on warm summer days, wearing a cooling vest (Beenakker et al., 2001; Nilsagård et al., 2006) or whole-body cryotherapy (E. Miller et al., 2016) has been recommended.

It must be noted, however, that no clear successes have been achieved so far. Some of the treatment approaches presented here as examples show controversial results or have been tested with very small sample sizes. Identifying the most appropriate treatment strategy is challenging due to the complex pathogenesis and heterogeneous set of fatigue symptoms. Therefore, the development of alternative approaches that directly attempt to modify the maladaptive cortico-striato-thalamo-cortical network represent new and promising approaches.

## 1.4 Transcranial electrical stimulation

Transcranial electrical stimulation (tES) is a non-invasive, non-pharmacological technique that can directly manipulate maladaptive neuronal activity. It allows for the direct observation of behavioral changes and, therefore, the investigation of causal relationships. Transcranial direct current stimulation (tDCS) and transcranial alternating current stimulation (tACS) are the most commonly used techniques. In both, a weak

current is applied via two electrodes, at least one of which is attached to the scalp. In tDCS, the current is constantly applied over a certain period of time. In contrast, in tACS, it is applied rapidly in a sinusoidally alternating manner in order to modulate brain activity via neuronal entrainment (Fertonani & Miniussi, 2017).

Both techniques have only minor side effects. These include tingling, itching, or redness under the stimulation electrodes. Mild headaches and more severe side effects are rarely reported after the stimulations (Cohen Kadosh et al., 2012). In 2017, Antal et al. (2017) published guidelines for the use of tES in research studies. Following these guidelines will ensure the safety and harmlessness of stimulation. In recent years, these methods have increasingly become the focus of therapeutic application for severe neurological diseases due to their simple application and cost-effectiveness (Cohen Kadosh et al., 2012).

Another advantage that tES offers is the possible application of a placebo stimulation. Thus, Nitsche and Paulus (2000) explained that a successful stimulation has lasting effects only when it lasts longer than three minutes. It is unlikely that stimulation times below this time window will produce long-term effects beyond the duration of the stimulation. As a result, in the control group, the stimulation will be turned on for one minute and then turned off. A process also called sham stimulation. Consequently, the tingling paresthesias typically associated with stimulation are also experienced by the control group, making it almost impossible to detect the stimulation condition (Ambrus et al., 2012; Gandiga et al., 2006).

### 1.4.1 Transcranial direct current stimulation

During tDCS, a constant current is applied, resulting in a shift of the resting membrane potential. One electrode is called the anode, and the other is called the cathode. During anodal stimulation, the current flows from the anode to the cathode, whereas during cathodal stimulation, the current flows from the cathode to the anode (Reed & Cohen Kadosh, 2018). For many years, the simple logic has been that the resting membrane potential is lowered during anodal stimulation, which in turn leads to increased excitability. Contrary, during cathodal stimulation, the potential is increased, thus reducing cortical responsiveness (Fertonani & Miniussi, 2017; Nitsche & Paulus, 2000; Reed & Cohen Kadosh, 2018). The actual relationship, however, turns out not to follow this simple logic. Thus, this dichotomy is more evident in studies on the effects on motor

functions, whereas in studies on cognitive functions, results also indicate increased activity after cathodal stimulation (Jacobson et al., 2012). It appears that these controversies arise from the fact that tDCS' effects depend on various factors. On the one hand, the effects depend on the polarity, the current intensity, the resulting current density as well as the stimulation duration. On the other hand, more recent findings have shown that effects also depend on individual parameters such as electrode-to-cortex distance, cerebrospinal fluid thickness, as well as the orientation of pyramidal neurons (Bikson et al., 2019; Mosayebi-Samani et al., 2021).

In contrast to the short-term shift in resting membrane potentials, tDCS-induced prolonged changes in neuronal activity are based on the principle of long-term potentiation. The repetitive action potentials enhance synaptic transmission as a result. As calcium ions accumulate, the density of AMPA receptors on the postsynaptic membrane increases, resulting in stronger coupling between neurons (Bhattacharya et al., 2022). Indeed, studies have reported increased intracellular calcium and cAMP levels after prolonged tDCS (Hattori et al., 1990). In addition, further studies have shown that tDCS effects are enhanced after the administration of D-cycloserine (an NMDA receptor agonist) and absent with NMDA receptor blockade (Monte-Silva et al., 2013; Nitsche et al., 2004).

### 1.4.2 Application in fatigue therapy

TDCS has been investigated several times to restore the altered neuronal activity in MS-related fatigue and fatigability. In the following, the studies that have investigated the effects of tDCS on subjective trait fatigue will be presented. In the last few years, there have also been first attempts to use tES for the treatment of fatigability. However, as these studies will be presented in detail later in this thesis, they will not be described in this chapter.

Based on the pathogenesis described for MS-related fatigue, tDCS regions were primarily directed at the cortico-striato-thalamic-cortical network. The majority of the studies were conducted over a period of several days, and fatigue symptoms were assessed before and after the stimulations using self-report questionnaires. Fatigue symptoms were improved after five stimulations of the primary motor cortex (Ferrucci et

al., 2014; Workman et al., 2020) or the somatosensory cortex (Cancelli et al., 2018; Tecchio et al., 2014; Tecchio et al., 2015). Fatigue symptoms decreased by up to 30% on average and persisted weeks after the stimulations. Most studies, however, examined the dorsolateral prefrontal cortex (DLPFC; Ayache et al., 2016; Ayache et al., 2017; Chalah et al., 2020; Chalah, Lefaucheur, & Ayache, 2017; Chalah, Riachi, et al., 2017; Charvet et al., 2018; Saiote et al., 2014). Likewise, fatigue symptoms decreased by up to 48 % (Chalah, Riachi, et al., 2017). Saiote et al. (2014) reported that fatigue symptoms decreased after the stimulations, but this occurred in both the verum and sham groups. Further data analysis revealed a positive correlation between lesion load in the DLPFC and stimulation effect. Accordingly, pwMS with higher lesion load benefited more from tDCS. A study by Charvet et al. (2018) conducted a study in which patients performed repetitive stimulations at their own homes. The results showed that stimulation combined with cognitive training positively affected fatigue symptoms compared to subjects receiving training alone. Furthermore, they reported that fatigue scores decreased more with 20 stimulations rather than ten stimulations. Two single-case studies similarly demonstrated that repetitive stimulation led to cumulative improvements (Ayache et al., 2017; Chalah, Lefaucheur, & Ayache, 2017) .

# 2 General Aim of this Thesis

With the preceding introduction, I have drawn attention to the current obstacles in fatigue research. Thus, the present concept of fatigue is purely subjective. The diagnosis lacks reliable and valid objective parameters hindering the validation of effective therapy options. Therefore, this thesis aims to (i) to complement the subjective fatigue diagnosis with behavioral and electrophysiological parameters and (ii) to investigate the effects of frontal tDCS on fatigue and fatigability in both healthy subjects as well as pwMS. As such, it intends to evaluate an alternative therapeutic strategy to alleviate one of the most common and severe symptoms of MS. This thesis will include three projects, in which I aim to comprehensively review the current literature (Project A) and investigate fatigue- and fatigability-related changes on electrophysiological parameters as well as their modulation by tDCS (Project B + C). An overview of the experimental settings of this thesis is shown in Table 1.

**Project A**

In Project A, I aim to review the previous literature on MS-related fatigue and fatigability and summarize the previous findings on objective parameters. Furthermore, I aim to discuss the relationship between subjective and objective parameters and to present studies on tES effects on fatigability.

**Project B**

In Project B, I aim to investigate the effects of repetitive tDCS on fatigability in pwMS.

As I have already described, several studies have shown positive effects of repetitive stimulation on subjective fatigue symptoms. In addition, some studies have demonstrated an improvement in fatigability symptoms after a single stimulation session in healthy controls (McIntire et al., 2014; McIntire et al., 2017; Nelson et al., 2014) and pwMS (Fiene et al., 2018). However, those studies found no improvement in subjective fatigue and no correlation between objective improvements and subjective feelings.

Therefore, I hypothesize that repetitive tDCS will positively affect subjective fatigue and fatigability. These positive effects will lead to objectively measurable differences in the verum group compared to the control group. Subjective trait fatigue will be operationalized via the WEIMuS questionnaire, subjective state fatigue via Numerical Rating Scales (NRS) and fatigability via the change in reaction time variability and P300 peak amplitudes.

**Project C**

Project C will focus on the systematic investigation of behavioral and electrophysiological fatigability parameters.

Thus, several studies have repeatedly demonstrated oscillatory brain activity changes during the performance of an exhaustive task (Clayton et al., 2015; Craig et al., 2012; Wascher et al., 2014). In addition, disrupted prepulse inhibition (PPI) and reduced sensory gating suppression have been demonstrated in healthy subjects as a result of fatigability (Aleksandrov et al., 2016; van der Linden et al., 2006). In the three studies of Project C, subjective trait fatigue in pwMS will be operationalized via the WEIMuS questionnaire, subjective state fatigue via visual analog scales (VAS), and fatigability via the change in frontomedial theta and occipital alpha power (Study C1 + C2), PPI and sensory gating ratios (Study C1 + C3), and reaction time variability (Study C2 + C3).

In Study C1, I will investigate fatigability-related changes in frontomedial theta and occipital alpha power as well as PPI and sensory gating ratios in healthy subjects after a 90-minute exhaustive task. Additionally, I will examine the effects of tDCS on the parameters compared to a control group. I hypothesize that fatigability will increase subjective fatigue ratings, as well as oscillatory brain activity. In addition, increased fatigability will result in reduced PPI and sensory gating ratios. Lastly, I hypothesize that anodal tDCS will counteract fatigability development. As a result, spectral measures and gating indices will be less affected.

In Study C2, I aim to investigate oscillatory brain wave activity changes and their relationship with subjective fatigue in pwMS. PwMS and HC will perform a 30-minute exhaustive task. Frontomedial theta and occipital alpha power will be assessed in resting state EEG as well as during the performance of the task. I hypothesize that pwMS

will experience greater fatigability compared to HC. This will lead to a more significant increase in VAS ratings, reaction time variability, and brain wave activity.

In Study C3, I aim to investigate whether PPI ratios and sensory gating suppression are objective indicators of cognitive fatigue in pwMS. Therefore, PPI and sensory gating ratios will be assessed before and after a 30-minute exhaustive task. I hypothesize that gating will be reduced in fatigued pwMS and that it will further be disrupted after the fatiguing task.

**Table 1.** *Overview of experimental designs.*

| | **Study sample** | | **Aim of the study** | **paradigms and methods** |
|---|---|---|---|---|
| | MS sample | HC sample | | |
| **PROJECT A:** | | | | |
| **Review article** | | | Integrate previous literature on cognitive fatigue and fatigability in MS, propose a unified taxonomy, and discuss evidence for the therapeutic potential of tES | |
| **PROJECT B:** | | | | |
| **Study B1** | 18 | - | Investigate the effects of repetitive tDCS on cognitive fatigue and fatigability | Serial Reaction Time Task, Auditory Oddball Paradigm, Numerical Rating Scales<br>Stimulation: 1.5 mA, 8 x 30 minutes, DLPFC |
| **PROJECT C:** | | | | |
| **Study C1** | - | 40 | Investigate PPI, P50 gating, alpha-, and theta-power as objective fatigability markers and investigate the potential of tDCS as a fatigability intervention | PPI, P50 sensory gating, 90 minutes AX-CPT task, VAS scales<br>Stimulation: 1.5 mA, 30 minutes, DLPFC |
| **Study C2** | 21 | 21 | Investigate oscillatory brain activity changes with time on task | Resting state EEG, CPT task, VAS scales |
| **Study C3** | 18 | 20 | Investigate the potential of PPI and P50 gating as fatigue and fatigability markers in MS | PPI, P50 sensory gating, 30 minutes AX-CPT task, VAS scales |

*AX-CPT,* AX- continuous performance task: *DLPFC,* dorsolateral prefrontal cortex; *HC,* healthy controls; *MS,* multiple sclerosis; *PPI,* prepulse inhibition; *tES,* transcranial electrical stimulation; *tDCS,* transcranial direct current stimulation; *VAS,* visual analog scales

# 3 Studies

## 3.1 Cognitive Fatigue in Multiple Sclerosis: An Objective Approach to Diagnosis and Treatment by Transcranial Electrical Stimulation

*A modified version of this chapter has been published as: Linnhoff, S., Fiene, M., Heinze, H.-J., & Zaehle, T. (2019). Cognitive Fatigue in Multiple Sclerosis: An Objective Approach to Diagnosis and Treatment by Transcranial Electrical Stimulation. Brain Sciences, 9(5). https://doi.org/10.3390/brainsci9050100*

### 3.1.1 Abstract

Cognitive fatigue is one of the most frequent symptoms in multiple sclerosis (MS), associated with significant impairment in daily functioning and quality of life. Despite its clinical significance, progress in understanding and treating fatigue is still limited. This limitation is already caused by an inconsistent and heterogeneous terminology and assessment of fatigue. In this review, we integrate previous literature on fatigue and propose a unified schema aiming to clarify the fatigue taxonomy. With special focus on cognitive fatigue, we survey the significance of objective behavioral and electrophysiological fatigue parameters and discuss the controversial literature on the relationship between subjective and objective fatigue assessment. As MS-related cognitive fatigue drastically affects quality of life, the development of efficient therapeutic approaches for overcoming cognitive fatigue is of high clinical relevance. In this regard, the reliable and valid assessment of the individual fatigue level by objective parameters is essential for systematic treatment evaluation and optimization.

Transcranial electrical stimulation (tES) may offer a unique opportunity to manipulate maladaptive neural activity underlying MS fatigue. Therefore, we discuss evidence for the therapeutic potential of tES on cognitive fatigue in people with MS.

### 3.1.2 Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system that leads to demyelination and atrophy of brain cells and has a profound impact on motor functioning and cognition. Worldwide the median prevalence is 33 per 100.000 people suffering from MS, with women being twice as often affected than men (Oh et al., 2018). MS is a very diverse disease with heterogeneous clinical symptoms. Depending on the area of inflammation and resulting lesions, various phenotypically different neurological deficits may occur. Among frequently reported deficits, fatigue remains one of the most common and challenging symptoms in MS affecting up to 75% of patients (Fisk et al., 1994; Kos et al., 2008). The syndrome includes a lack of motivation, an overall feeling of exhaustion as well as behavioral performance decrements, and is the main reason for early retirement in people with MS (Simmons et al., 2010). The exact pathogenic mechanisms underlying MS fatigue are yet not fully understood. Particularly three influential core hypotheses have been proposed. Accordingly, fatigue has been related to (1) neuroimmune dysregulation based on increased levels of inflammatory mediators such as interferon or interleukin, (2) neuroendocrine dysfunction resulting in hyperactivation of the hypothalamo–pituitary–adrenal axis, and (3) demyelination, cortical lesions, and functional brain abnormalities within various cortical and subcortical brain regions (see Ayache & Chalah, 2017 for a review of studies on the pathogenesis of MS fatigue). The latter hypothesis is supported by a large number of neuroimaging studies proposing a malfunctioning cortico–striato–thalamo–cortical network, the so called fatigue circuit, underlying MS fatigue (Chalah et al., 2015; Chaudhuri & Behan, 2000). Hence, various previous research demonstrated relations between subjective trait-fatigue and structural and functional abnormalities in the frontal regions (Pardini et al., 2010; Riccitelli et al., 2011; Rocca et al., 2014; Roelcke et al., 1997; Sepulcre et al., 2009), parietal regions (Colombo et al., 2000; Pellicano et al., 2010; Sepulcre et al., 2009), corpus callosum (Gobbi et al., 2014; Yaldizli et al., 2011; Yaldizli et al., 2014), basal ganglia (Inglese et al., 2007; Niepel et al., 2006; Roelcke et al., 1997; Téllez et al., 2008), and thalamus (Inglese et al., 2007; Niepel et al., 2006).

Regarding its diagnosis, the fatigue construct has been divided into a motoric, psychosocial, and cognitive dimension (Fisk et al., 1994). In this review, we will focus specifically on the assessment and therapy of the latter dimension. Cognitive fatigue significantly impairs daily life and is just as debilitating to people with MS as motoric fatigue. However, the concept of cognitive fatigue is still only poorly understood. According to the multidimensional nature of MS fatigue, various definitions exist in the current literature. The MS Council (1998) defines MS fatigue in general as a "subjective lack of physical and/or mental energy that is perceived by the individual or caregiver to interfere with usual and desired activities" (Multiple Sclerosis Council for Clinical Practice Guidelines, 1998, p. 2), which specifically emphasizes the current subjective understanding of the syndrome. As a result, multiple self-report questionnaires assessing the severity of fatigue, such as the Fatigue Severity Scale (FSS; Krupp et al., 1989), the Fatigue Impact Scale (FIS; Fisk et al., 1994), the Modified Fatigue Impact Scale (MFIS; MS Council, 1998), the Fatigue Scale for Motor and Cognitive Functions (FSMC; Penner et al., 2009), or the Wuerzburg Fatigue Inventory for Multiple Sclerosis (WEIMuS; Flachenecker et al., 2006) have been developed. While FSS is only a one-dimensional questionnaire, the other four evaluate distinct fatigue dimensions, including cognitive fatigue. Importantly, although these questionnaires are extensively used to diagnose cognitive fatigue, they exclusively assess the subjective experience of people with MS. Yet, subjectively assessed parameters are retrospective statements and therefore mood-sensitive and subject to psychological errors, such as regression to the mean or recall bias which reduce their diagnostic accuracy (Fiene et al., 2018). Additionally, these questionnaires show low correlations among each other and heterogeneous associations to patients' functional impairment, disease duration, or cognitive deficits (Barak & Achiron, 2006; Flachenecker et al., 2002; Niepel et al., 2006; Pellicano et al., 2010). Thus, for comprehensive clinical diagnostics of cognitive fatigue, assessment of subjective exhaustion needs to be complemented by the objectively measurable impact of fatigue on patients' functioning. As suggested by Holtzer et al. (2011), this objective cognitive fatigue can be assessed as behavioral consequences of "an executive failure to maintain and optimize performance over acute but sustained cognitive effort" as this will result "in performance that is lower and more variable than the individual's optimal ability" (Holtzer et al., 2011, p. 108). Hence, according to its definition cognitive fatigue must be operationalized as strong performance decrements in cognitive demanding tasks over

time, rather than as current performance at only one measurement time point, as the latter might only reflect the level of overall cognitive impairment.

The utilization of a unified taxonomy and its precise use in research communication is of particular importance for future progress in MS-related fatigue research. In Figure 4, we propose a generally valid fatigue taxonomy. Summarizing former suggestions, fatigue can be subdivided into physical, psychosocial, and cognitive fatigue (Fisk et al., 1994). While psychosocial fatigue is only subjectively measurable, physical and cognitive fatigue can be assessed subjectively as well as objectively. Specifically, subjective cognitive fatigue refers to an ongoing perceived feeling of exhaustion. Objective cognitive fatigue - hereafter referred to as fatigability - describes a performance decline during cognitive tasks, measurable through the change in cognitive performance relative to a baseline (Kluger et al., 2013). Subjective and objective cognitive fatigue can be further subdivided. Subjective fatigue divides into a trait and a state component. Trait-fatigue refers to a global status of the patient that changes slowly over time, while state-fatigue means the change in subjectively perceived fatigue level over time (Genova et al., 2013). Accordingly, subjective trait-fatigue can be evaluated through self-questionnaires and subjective state-fatigue through visual analog scales (VAS) or numerical rating scales.

In contrast, objective fatigue (fatigability) is per definition state-dependent and enables an objective assessment by behavioral or electrophysiological parameters that will be explained in detail in the following section. Thus, the proposed concept of cognitive fatigue implies that it can be studied both qualitatively as a subjective phenomenon and quantitatively as an objective phenomenon.

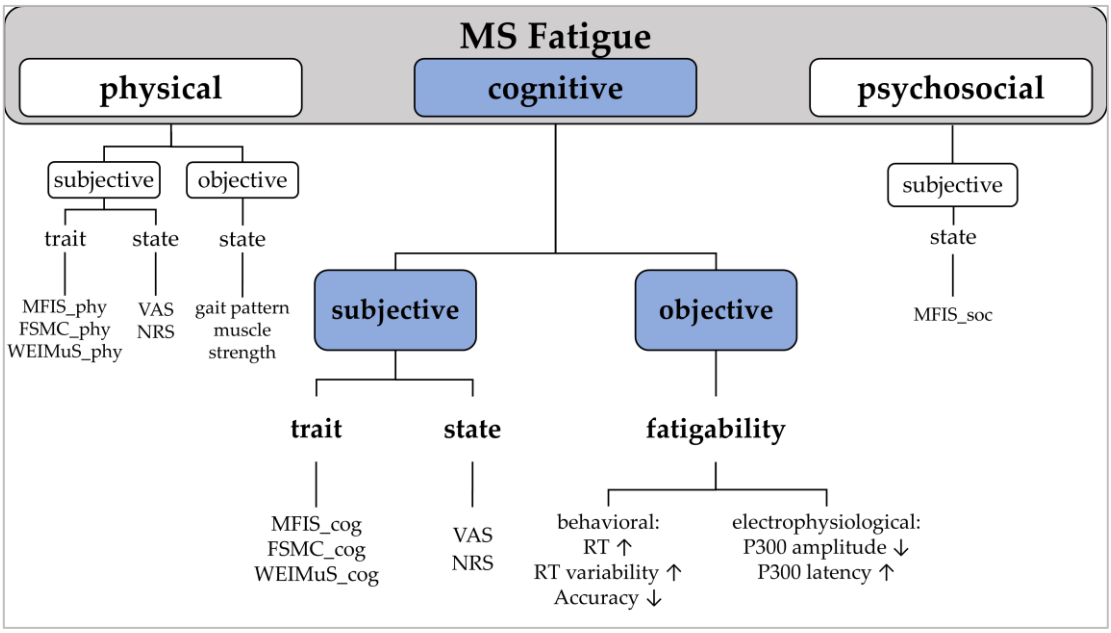


**Figure 4.** Fatigue Classification. *MFIS,* Modified Fatigue Impact Scale; *MS,* multiple sclerosis; *FSMC,* Fatigue Scale for Motoric and Cognitive Functions; *WEIMuS,* Wuerzburg Fatigue Inventory for Multiple Sclerosis; *VAS,* Visual Analog Scale; *NRS,* Numerical Rating Scale; *RT,* reaction time; ↑, increase; ↓, decrease.

### 3.1.3 Search strategies

In order to give an exhaustive overview of the literature, we searched for relevant studies in English and German languages addressing MS fatigue on electronic databases (i.e., PubMed, Scopus, and Cochrane database) until the end of January 2019. The following research terms and cross-combinations of the terms were used: “multiple sclerosis” or “MS”, “fatigue”, “fatigability”, “cognitive fatigue”, “objective fatigue”, “performance decrement”, “time on task”, “noninvasive brain stimulation”, and “transcranial direct current stimulation” or “tDCS” and “transcranial alternating current stimulation” or “tACS”. Further, we also scanned the references of the selected studies in order to look for additional relevant sources.

### 3.1.4 Objective Measurement of Cognitive Fatigue

To overcome the purely subjective character of fatigue diagnostics, recent research focused on the investigation of objective diagnostic measures of fatigability in people

with MS. The methods to operationalize fatigability as performance reduction with time-on-task can be divided into four approaches (DeLuca, 2005). The first approach is to investigate fatigability over a prolonged period of time, in which the subjects perform the test paradigm several times in a row and performance changes are compared to a baseline. Applying this approach, some studies reported evidence for a fatigue-related performance decline (Bailey et al., 2007; Claros-Salinas et al., 2010; Fiene et al., 2018), while others did not (Andreasen et al., 2010; Beatty et al., 2003; Bruce et al., 2010; Johnson et al., 1997). The second and third approach define fatigability as a pre-to-post performance decline in a specific task A while inducing fatigue by mental (second approach) or physical exertion (third approach) in a task B in between. However, evidence in support of these approaches is rare and inconsistent (Krupp & Elkins, 2000; Neumann et al., 2014). The fourth and most promising method is to measure fatigability during sustained mental effort and to compare the performance at the beginning of a cognitively demanding task with performance level at the end. Using this approach, fatigability has been repeatedly demonstrated (Aldughmi et al., 2017; Bryant et al., 2004; Chinnadurai et al., 2016; Gossmann et al., 2014; Kos et al., 2004; Kujala et al., 1995; Moore et al., 2017).

Fatigue has been shown to become most prominent during sustained attention tasks that depend on a high level of endogenous attention. Accordingly, subjective fatigue shows strong relations to performance decline in alertness and vigilance tasks (Aldughmi et al., 2017; Claros-Salinas et al., 2010; Claros-Salinas et al., 2013; Fiene et al., 2018; Gossmann et al., 2014; Hanken, Bosse, et al., 2016; Kujala et al., 1995; Neumann et al., 2014). In contrast, it does not impair memory performance, language, or visuospatial processing (Andreasen et al., 2010; Bruce et al., 2010; Heesen et al., 2010; Krupp & Elkins, 2000) and shows only weak associations with performance decline in tasks on processing speed (Andreasen et al., 2010; Beatty et al., 2003; Bryant et al., 2004; Johnson et al., 1997; Kluckow et al., 2016; Kos et al., 2004; Schwid et al., 2003) and working memory (Bailey et al., 2007; Lehmann et al., 2012; Walker et al., 2012). According to Hanken, Eling, and Hildebrandt (2015), only alertness or vigilance tasks require maintained intrinsic attention over a prolonged period of time that can easily be distracted by interoceptive events or mind wandering, which can result in cognitive fatigability. This relation is further supported by an overlap of neural alterations in the fatigue circuit and

brain regions involved in attentional processing (Calabrese et al., 2010; Derache et al., 2013; Engstroem et al., 2013; Hidalgo de Cruz et al., 2018; Yaldizli et al., 2014).

In the following, we will present a series of objective parameters that have been proven to be suitable surrogate markers for assessing fatigability. Table 2 presents an overview of studies investigating objective cognitive fatigue in people with MS, sorted by the used approaches to measure fatigability.

**Table 2.** *Overview of studies investigating objective cognitive fatigability in people with multiple sclerosis (MS).*

| Reference | Parameter | Sample Size | EDSS Score | Duration of MS (in years) | Conceptualization | Fatigability | Correlation with Subjective Fatigue |
|---|---|---|---|---|---|---|---|
| **Cognitive fatigue over an extended time** | | | | | | | |
| Andreasen et al. (2010) | Processing speed | 60 MS (all RR), 18 HC | PF: 3.0 (1-3.5) [b]<br>SF: 2.5 (2-3.5) [b]<br>NF: 2 (1.5-3.5) [b] | PF: 5.0 (1-14) [b]<br>SF: 3.5 (0-16) [b]<br>NF: 3.0 (0-9) [b] | Processing speed across two testing blocks | No: processing speed improved in second testing block | Yes: negative correlation between subjective trait-fatigue and cognitive performance |
| Bailey et al. (2007) | RT, Accuracy | 14 MS (all PP + SP), 17 HC | 7.7 (0.4) [a] | 27.2 (8-59) [c] | Performance in 0-back (attention) and 1-back task (working memory) | Yes: accuracy decreased over time | Between subjective state-fatigue and fatigability |
| Beatty et al. (2003) | Processing speed | 17 MS (13 RR, 4 SP), 12 HC | 2.9 (2.3) [a] | 14.2 (7.4) [a] | Performance in cognitive tests (list recall, letter-number sequence, SDMT, PASAT) before and after workday | No: no performance decline from first to second testing block | No: no correlation between subjective state-fatigue and cognitive performance after workday |
| Bruce et al. (2010) | RT, RT variability | 87 MS (70 RR, 17 SP), 24 HC | 4.6 (1.6) [a] | 10.9 (7.9) [a] | Performance across three blocks of CARB | No: shorter RT and smaller variability over time | Yes: positive correlation between subjective trait-fatigue and cognitive performance |
| Claros-Salinas et al. (2010) | RT | 20 MS, 76 HC, 22 stroke | - | 8.2 (7.2) [a] | Performance in three TAP subtests at three different time points of the day | Yes: cognitive performance decreased over time only in MS patients | Not mentioned |
| DeLuca et al. (2008) | RT | 15 MS (12 RR, 3 PP), 15 HC | - | 6.4 (4.9) [a] | Performance across four blocks of modified SDMT | No: faster RT over time | Not mentioned |

**Table 2.** *Cont.*

| Reference | Parameter | Sample Size | EDSS Score | Duration of MS (in years) | Conceptualization | Fatigability | Correlation with Subjective Fatigue |
|---|---|---|---|---|---|---|---|
| Fiene et al. (2018) | RT, P300 amplitude and latency | 15 MS (14 RR, 1 SP) | 3.5 (1.9) [a] | 9.6 (8.6) [a] | Performance across three blocks of SRT and auditory oddball paradigm | Yes: increasing RT, shorter amplitude, and longer latencies of P300 over time | Yes: correlation between subjective state-fatigue and fatigability (negative with P300 amplitude and positive with latency) |
| Huolman et al. (2011) | Processing speed, RT | 15 MS (all RR), 13 HC | 1.5 (0.9) [a] | 4.2 (3.6) [a] | Performance of the last 20 items across four blocks of a modified version of the PVSAT | Yes: group differences increased over time | Not mentioned |
| Johnson et al. (1997) | Processing speed | 15 MS, 15 CPS, 15 MD, 15 HC | 1.8 (1.2) [a] | - | PASAT performance across four testing blocks | No: performance unchanged across blocks | Not mentioned |
| Sandry et al. (2014) | RT, Accuracy | 32 MS (24 RR, 1 PP, 3 SP, 1 PR), 24 HC | AI*: 2.4 (2.5) [a] | 11.9 (7.1) [a] | Task performance (processing speed and working memory task) across four testing blocks | No: RT improved across blocks, no changes in accuracy | No: no correlation between subjective state-fatigue and cognitive performance across blocks |
| **Cognitive fatigue after challenging mental or physical exertion** | | | | | | | |
| Claros-Salinas et al. (2013) | RT | 32 MS (20 RR, 2 PP, 10 SP), 20 HC | 3.6 (1.6) [a] | 7.7 (5.4) [a] | Performance in TAP subtests before and after physical and cognitive load for 2.5 hours | Yes: people with MS showed a significant increase in RT after cognitive load | Yes: positive correlation between subjective trait as well as state-fatigue and fatigability |

**Table 2.** *Cont.*

| Reference | Parameter | Sample Size | EDSS Score | Duration of MS (in years) | Conceptualization | Fatigability | Correlation with Subjective Fatigue |
|---|---|---|---|---|---|---|---|
| Jennekens-Schinkel et al. (1988) | RT | 39 MS (20 RR, 19 PP+SP), 25 HC | 3.5 (0-7) [c] | 12.0 (1-48) [c] | Performance in SRT before and after neuropsychological assessment for four hours | No: no group differences in task-related performance decline | Not mentioned |
| Krupp & Elkins (2000) | Neuropsycho-logical test battery | 45 MS (24 RR, 8 PP, 13 SP), 14 HC | 3.8 (1.7) [a] | - | Performance in neuropsychological test battery before and after a cognitive demanding task | Yes: performance of people with MS worsened after cognitive task | Not mentioned |
| Neumann et al. (2014) | RT | 30 MS (23 RR, 1 PP, 6 SP), 15 HC | F: 3.8 (1.2) [a]<br>NF: 3.7 (0.6) [a] | F: 9.9 (6.7) [a]<br>NF: 13.6 (6.8) [a] | Performance in TAP alertness test before and after cognitive load and after a one hour resting time | Yes: increased RT in MS group after cognitive load; after rest, RT returned to baseline in most patients | Yes: positive correlation between subjective trait-fatigue and cognitive performance |
| Paul et al. (1998) | Accuracy, memory performance | 39 MS, 19 HC | AI: 4.1 (2.5) [a] | 12.2 (4.8) [a] | Performance in Word List Learning Task and vigilance test before and after a cognitive work battery that lasted 30 min | No: neither patients nor controls showed changes in cognitive performance after 20 min task | Not mentioned |
| Spiteri et al. (2017) | RT | 40 MS (25 RR, 2 PP, 13 SP), 22 HC | 3.5 (1.5) [a] | 14.1 (8.8) [a] | Performance in alertness test before and after a cognitive demanding task (n-back) | Yes: patients responded slower and with greater variability after n-back task | No: no correlation between subjective trait as well as state-fatigue and cognitive performance |

**Table 2.** *Cont.*

| Reference | Parameter | Sample Size | EDSS Score | Duration of MS (in years) | Conceptualization | Fatigability | Correlation with Subjective Fatigue |
|---|---|---|---|---|---|---|---|
| **Cognitive fatigue during sustained mental effort** | | | | | | | |
| Berard et al. (2018) | Processing speed | 32 MS (all RR), 32 HC | 1.8 (2.2) [a] | 4.4 (3.1) [a] | Performance in first third versus last third of PASAT | Yes: poorer performance in last third of PASAT | No: no correlation between subjective trait-fatigue and fatigability |
| Bryant et al. (2004) | Processing speed | 56 MS, 39 HC | - | SG1: 5.8 (1.6) [a]<br>SG2: 10.6 (1.8) [a] | Performance in first versus second half of each of four PASAT testing blocks | Yes: percent dyads declined earlier in time in MS subgroup | No: no correlation between subjective trait-fatigue and cognitive performance |
| Cehelyk et al. (2019) | RT | 21 MS (19 RR, 2 SP) | 3.5 (1.6) [a] | 13.3 (8.7) [a] | Performance in first versus fourth quarter of Blocked Cyclic Naming Task | Yes: RT increased from first to fourth quarter | Yes: positive correlation between subjective state-fatigue and fatigability |
| Chinnadurai et al. (2016) | Processing speed, P300 | 50 MS (36 RR, 2 PP, 12 SP), 50 HC | 4.6 (1.9) [a] | 6.0 (7.4) [a] | Performance in 60 and 180 sec version of Stroop Task, SDMT, serial addition task and ratio between first and last 50 items in P300 oddball paradigm | Yes: performance decline and increasing P300 latencies in last 50 items only in people with MS | Not mentioned |
| Crivelli et al. (2012) | RT | 27 MS (all RR), 27 HC | 1.03 (0.8) [a] | 0.7 (0.7) [a] | Performance in third compared to first block of three attentional network tests (alertness, orienting, executive control) | No: performance improved over time | Not mentioned |

**Table 2.** *Cont.*

| Reference | Parameter | Sample Size | EDSS Score | Duration of MS (in years) | Conceptualization | Fatigability | Correlation with Subjective Fatigue |
|---|---|---|---|---|---|---|---|
| DeLuca et al. (2008) | RT, Accuracy | 15 MS (12 RR, 3 PP), 15 HC | - | 6.4 (4.9) [a] | Performance in second compared to first half in each of four blocks of modified SDMT | No: both groups responded faster in second half of each block | Not mentioned |
| Gossmann et al. (2014) | Accuracy | 31 MS (all RR), 10 HC | 3.6 (2.1) [a] | 10.4 (9.2) [a] | Omissions in second half compared to first half of a 30 min auditory vigilance test | Yes: only in MS group performance declined significantly during the task | Yes: positive correlation between subjective state-fatigue and fatigability |
| Hanken et al. (2016) | RT | 46 MS (18 RR, 28 PP+SP) | LF: 3.7 (1.8) [a]<br>HF: 4.7 (1.1) [a] | LF: 13.5 (8.8) [a]<br>HF: 10.9 (7.8) [a] | Performance in first 5 min compared to last 5 min of a 20 min visual vigilance task | Yes: RT increased with time-on-task | Not mentioned |
| Kluckow et al. (2016) | Processing speed | 36 MS (all RR), 36 HC | 1.9 (1.2) [a] | 2.8 (6.6) [a] | Performance in PASAT during the last 20 items compared to first 20 items and performance change in TVA from first to fourth block | Yes: processing speed of MS group declined in second half of TVA (especially in high-fatigue patients) | Not mentioned |
| Kos et al. (2004) | Processing speed | 50 MS, 21 HC | 6.4 (1.2) [a] | - | Performance in the first ten items compared to the last ten items in PASAT | Yes: 21.1 % performance decline in MS group | No: no correlation between subjective trait-fatigue and fatigability |
| Kujala et al. (1995) | RT, Accuracy | 45 MS (22 RR, 17 PP, 6 SP), 35 HC | CP: 5.0 (1.8) [a]<br>CD: 5.5 (1.3) [a] | CP: 8.7 (5.9) [a]<br>CD: 8.7 (6.0) [a] | Performance in visual vigilance test over 15 min | Yes: slower RT with time-on-task; the cognitively preserved MS group also showed decline in accuracy | Not mentioned |

**Table 2.** *Cont.*

| Reference | Parameter | Sample Size | EDSS Score | Duration of MS (in years) | Conceptualization | Fatigability | Correlation with Subjective Fatigue |
|---|---|---|---|---|---|---|---|
| Lehmann et al. (2012) | RT, Accuracy | 42 MS (all RR), 11 HC | F: 2.8 (1.4) [a]<br>NF: 4.3 (2.7) [a] | - | Performance decline from first to second half of a 10 min 2-back task | No: no task-related performance changes with time-on-task | Not mentioned |
| Schwid et al. (2003) | Processing speed | 20 MS (10 RR, 2 PP, 8 SP), 21 HC | 3.8 (1.5) [a] | - | Performance in first 20 items compared to last 20 items in PASAT | Yes: performance decline over time only in people with MS | Yes: correlation between subjective trait-fatigue and fatigability |
| Walker et al. (2012) | Processing speed | 70 MS (all RR), 70 HC | 1.8 (1.2) [a] | 4.4 (3.1) [a] | Performance during first compared to second half in PASAT and CTIP | Yes: ability of MS group to meet task demands declined over time | Yes: negative correlation between subjective trait-fatigue and fatigability |

* AI (Ambulatory Index): is based on a zero-to-nine-point scale and has been shown to be highly correlated with Expanded Disability Status Scale (EDSS) [72]. (a) mean (standard deviation); (b) median (range); (c) mean (range). Abbreviations: *AI,* Ambulatory Index; *CARB,* Computerized Assessment of Response Bias; *CD,* cognitively deteriorated subgroup; *CFS,* chronic fatigue syndrome; *CP,* cognitively preserved subgroup; *CTIP,* Computerized Test of Information Processing; *EDSS,* Expanded Disability Status Scale; *F,* fatigued subgroup; *HC,* healthy controls; *HF,* high fatigued subgroup; *LF,* low fatigued subgroup; *MD,* major depression; *MS,* multiple sclerosis; *NF,* non-fatigued subgroup; *PASAT,* Paced Auditory Serial Addition Test; *PF,* primary fatigued subgroup; *PP,* primary progredient MS form; *PR,* progressive relapsing MS form; *PVSAT,* Paced Visual Serial Addition Test; *RR,* relapsing-remitting MS form; *RT,* reaction time; *SDMT,* Symbol Digit Modalities Task; *SF,* secondary fatigued subgroup; *SG,* subgroup; *SP,* secondary progredient MS form; *SRT,* Simple Reaction Time Task; *TAP,* Test Battery for Attentional Performance; *TVA,* Theory of Visual Attention.

#### 3.1.4.1 Behavioral Measures

Behaviorally, fatigability can be assessed through changes in reaction time, accuracy, and processing speed in simple alertness or vigilance tests over time. There are numerous studies showing increasing reaction times (Cehelyk et al., 2019; Claros-Salinas et al., 2010; Claros-Salinas et al., 2013; Fiene et al., 2018; Huolman et al., 2011; Krupp & Elkins, 2000; Kujala et al., 1995; Neumann et al., 2014) and decreasing accuracy (Bailey et al., 2007; Bryant et al., 2004; Chinnadurai et al., 2016; Gossmann et al., 2014) with time-on-task, mostly assessed by administering simple reaction time tests such as the alertness subtest of the Test Battery for Attentional Performance (TAP; Psychological Test Systems). Claros-Salinas et al. (2010) measured fatigue in the TAP alertness task at three different time points during the course of one day. While subjective state-fatigue increased diurnally in both participants with MS and healthy controls, cognitive performance decreased over the day only in the MS group. Furthermore, performance changes in the TAP alertness task were assessed at baseline and after 2.5 hours of physical and cognitive exertion. While healthy controls improved from first to second test administration, performance of the MS group decreased (Claros-Salinas et al., 2013). Similarly, Neumann et al. (2014) investigated fatigability by measuring the alertness level before and after participants performed a cognitively demanding task. The authors found increased reaction times after cognitive load only in people with MS, while reaction time remained unchanged in healthy controls. Thus, these studies indicate that objective cognitive fatigue parameters are well suited for assessing MS-related fatigue pathology.

It is noteworthy, however, that there are also studies showing no susceptibility of reaction time (Bailey et al., 2007; Jennekens-Schinkel et al., 1988; Lehmann et al., 2012) or accuracy performance (DeLuca et al., 2008; Paul et al., 1998) to cognitive fatigue. Therefore, some authors suggested finer-grained analytical methods like reaction time variability, which is defined as the standard deviation of correct response times or the coefficient of variation, which is calculated by dividing the standard deviation by the mean reaction time and thus avoiding confounding effects of group differences in mean reaction times (Bodling et al., 2012; Bruce et al., 2010). Cognitive fatigue may lead to occasional lapses in attention followed by higher reaction time variability even in the absence of a linear time-on-task decline (Bruce et al., 2010). Accordingly, analyses

accounting for individual variability might be more sensitive in diagnosing behavioral fatigability effects.

Besides reaction times, cognitive processing speed and working memory changes can further be indicators for cognitive fatigue declines. They are commonly assessed using the Paced Auditory Serial Addition Test (PASAT; Gronwall, 1977) or the Signal Digit Modalities Test (SDMT). Studies utilizing the PASAT to measure fatigability typically report a significant performance reduction from the first through the second half of the task (Bryant et al., 2004; Kos et al., 2004; Schwid et al., 2003; Walker et al., 2012). A more fine-grained analysis is the percent dyad score method suggested by Snyder et al. (2001). This score only counts the total number of two correct responses in a row proposed as a better estimate of performance correctness according to the intended task demands. In line with this assumption, one study showed that the total number of correct responses in the PASAT did not differ between participants with MS and healthy controls, while when examining percent dyad score, only the MS group showed pronounced susceptibility to cognitive fatigability (Bryant et al., 2004).

#### 3.1.4.2 Electrophysiological Measures

Recording of brain activation by electroencephalography (EEG) and event-related potentials (ERP) has been proven as a sensitive method for the objective assessment of neural alterations related to cognitive fatigue. Specifically, the P300 ERP is widely used as an index of cognitive functioning (Picton, 1992; Sutton et al., 1965). The component is generally evoked in an oddball paradigm when rare target stimuli are presented in a sequence of standard stimuli. The P300 amplitude is proposed to be proportional to the amount of attentional resources devoted to a given task, while P300 latency indicates processing speed (Polich, 2007).

Previous studies demonstrated longer latencies and smaller amplitudes of P300 component in people with MS (Piras et al., 2003; Polich et al., 1992). Pokryszko-Dragan et al. (2016) investigated changes in P300 and cognitive performance in patients and found prolonged latencies and reduced amplitude of P300 associated with increased subjective cognitive fatigue. Chinnadurai et al. (2016) conceptualized fatigability as the ratio between the processing of the first and last items in an ongoing oddball paradigm

and evaluated P300 alterations. As a result, participants with MS showed prolonged P300 latencies for the last 50 items compared to first 50 items. Regarding P300 amplitude, data revealed no significant difference between people with MS and healthy controls. In our recent interventional study (Fiene et al., 2018), patients with subjective fatigue performed three blocks of an auditory oddball paradigm to assess cognitive fatigability. The MS group that did not receive an intervention showed fatigability-related increased P300 latencies and decreased amplitudes with time-on-task.

#### 3.1.4.3 Sensory Gating Parameter

Sensory gating plays a key role in cognitive control and attention. It protects stimulus processing from interference caused by subsequent incoming information. Sensorimotor gating can be measured using prepulse inhibition (PPI). PPI means a reduced startle response to an intense stimulus when a low intensity stimulus (prepulse) is presented beforehand. van der Linden et al. (2006) investigated 20 healthy subjects that were randomly allocated to a fatigue or non-fatigue condition. Before and after a cognitively demanding task, PPI was evaluated. Results showed a significant reduction in PPI during cognitive fatigue state. Thus, induction of cognitive fatigue by a cognitively demanding task negatively affected sensorimotor gating. Additionally, the reduction in PPI correlated positively with subjective state-fatigue evaluated by VAS. Another sensory gating parameter is the event-related potential P50. The P50 is generally evoked using the auditory paired click paradigm, when one click sound is followed by a second click sound approximately 500 ms after the first one. The processing of the first stimulus suppresses processing of the second stimulus, thereby leading to decreased P50 amplitude to the second click. One study by Aleksandrov et al. (2016) examined the P50 before and after inducing cognitive fatigue by muscle load. Data showed that physical exertion significantly decreased or completely suppressed the sensory gating index. However, no study systematically investigated PPI and P50 changes through fatigability in people with MS so far. Whether the diagnostic value of sensory gating parameters shown in clinically non-significant fatigue in healthy subjects can be generalized to pathological MS fatigue needs to be further investigated.

Taken together, fatigability is best operationalized with sustained attention tasks measuring alertness or vigilance declines over time. Sustained attention tasks like

the TAP alertness subtest, PASAT, and SDMT have been proven to reliably lead to objectively measurable performance declines in people with MS. Other cognitive domains like memory, language, visuospatial processing, verbal learning, or working memory do not seem to be consistently affected by cognitive fatigue. Parameters that have been shown to represent objective indices for fatigability in people with MS are simple reaction time and accuracy, as well as the P300 ERP. Fatigability consistently leads to increasing reaction times, decreasing accuracies, and smaller amplitudes, as well as longer latencies in the P300 ERP component. Additionally, recent studies present finer-grained analyses (i.e., response time variability, coefficient of variant, or percent dyad score) as more sensitive measures of performance decline over time. To reliably measure clinically significant fatigability in people with MS, it is, however, important to differentiate between patients with and without fatigue. Fatigue-related strong performance deteriorations can otherwise not be distinguished from typical performance decrements over time that might also occur in healthy subjects. Common approaches to determine the clinical significance of fatigue symptoms are the definition of cut-off values on subjective fatigue questionnaires or the investigation of statistically significant differences in fatigability between patients and healthy controls (Kluger et al., 2013). Moreover, closer investigations on the relation between the level of objective cognitive fatigue and demographic characteristics of patients (e.g., disease duration or disability status) can help to understand the implications of this symptom for patients' daily functioning during the course of the disease. These aspects should be considered in future studies on cognitive fatigue.

### 3.1.5 Relationship between Objective and Subjective Fatigue

The relation between subjective fatigue and fatigability is still a topic of controversy. If cognitive fatigue affects task performance of people with MS, it should be paralleled by subjectively perceived fatigue. However, there are numerous studies showing no relationship between subjective and objective cognitive fatigue measures (Bailey et al., 2007; Beatty et al., 2003; Bryant et al., 2004; Deloire et al., 2006; Hulst et al., 2013; Karadayi et al., 2014; Morrow et al., 2009; Niino et al., 2014; Parmenter et al., 2003; Sandry et al., 2014; Sundgren et al., 2013). One reason for this observed divergence might be related to the high heterogeneity in the diagnostic scales used for assessing subjective cognitive fatigue. Additionally, in most studies, subjective fatigue questionnaires mainly

measure the trait component of fatigue by assessing the impact of fatigue on daily activities over the past weeks. However, since fatigability is defined as a performance decrement over time, it might be more related to changes in subjective state-fatigue during the course of a testing session. In the following, we discuss this aspect in more detail by differentiating between studies measuring subjective trait- or state-fatigue and correlating these values with overall mean performance or changes in performance.

Of those studies investigating the relationship between subjective trait-fatigue and mean cognitive performance during a fatiguing task, three found a positive correlation between FSS score and reaction time (Andreasen et al., 2010; Weinges-Evers et al., 2010) or P300 ERP (Pokryszko-Dragan et al., 2016), and another three studies reported a positive relation specifically between cognitive fatigue and reaction times (Bruce et al., 2010; Greim et al., 2007; Neumann et al., 2014). Two studies found no associations (Bryant et al., 2004; Morrow et al., 2009). Five studies examined the relationship between subjective trait-fatigue and fatigability indicated by performance decrement over time. While one study reported no association (Kos et al., 2004), three studies found a positive relationship (Aldughmi et al., 2017; Claros-Salinas et al., 2013; Walker et al., 2012), indicating slower processing speed and longer reaction times as the task progressed with greater subjective trait-fatigue. Schwid et al. (2003) found a significant correlation between the FSS questionnaire and performance change from the first to second half in the PASAT, but no correlations between performance decline and the MFIS cognitive fatigue subscale. Five studies especially examined subjective state-fatigue and their relationship to objective performance decline with time-on-task. Four studies found a positive relationship (Cehelyk et al., 2019; Claros-Salinas et al., 2013; Fiene et al., 2018; Gossmann et al., 2014), while only one study did not (Bailey et al., 2007). Hence, longer reaction times with time-on-task as well as more omissions in the second half were shown to be associated with a greater feeling of momentary exhaustion (Cehelyk et al., 2019; Claros-Salinas et al., 2013; Gossmann et al., 2014). Finally, in a recent study, we further revealed a positive association between subjective state-fatigue assessed by a VAS and P300 latency and a negative relation with P300 amplitude (Fiene et al., 2018).

Due to this heterogeneity in the literature, the relationship between subjective and objective fatigue measures still remains unclear. Assuming that in some patients, subjective and objective fatigue jointly appear, correlations might only be detectable

when choosing valid fatigue parameters. For objective fatigue measures, this might include the change in simple reaction time, accuracy, and ERPs. However, not only the choice of objective fatigue markers but also the reliable assessment of subjective fatigue changes over time is a challenging methodological factor. Likert rating scales are frequently used but have limited variability that hampers the detection of correlations with objective fatigability measures. Based on former studies, Hanken et al. (2014) proposed a theory inclining the subjective feeling of fatigue and the objectively measurable fatigability into one model. They proposed that subjective fatigue results from inflammation-induced sickness behavior and altered neural processing within interoceptive and homeostatic brain areas, including the insula, the anterior cingulate, and the hypothalamus. Via increased interoceptive interference, subjective fatigue might secondarily lead to objective fatigue symptoms in terms of measurable performance decrements. Importantly, the latter can even be exaggerated by cortical atrophy in the alerting system, thereby accounting for the relevance of the attention network contributing to the pathogenesis of objective cognitive fatigue. Hence, according to their model, objectively measurable performance changes can also exist independent of subjective fatigue due to cortical atrophy in the attention network, which might explain variability in correlations between subjective and objective fatigue in the current literature. These considerations demonstrate the importance of including both subjective and objective fatigue in a holistic fatigue concept and emphasize the use of a clear and unified taxonomy in future fatigue research.

### 3.1.6 Therapeutic Potential of tES for Cognitive Fatigue

As MS-related cognitive fatigue drastically affects a patient´s quality of life, the development of efficient therapeutic methods for overcoming fatigue is of high clinical relevance. Especially for a systematic treatment evaluation and optimization, a reliable and valid assessment of the individual fatigue level by objective parameters is essential. Transcranial electrical stimulation (tES) may offer a unique opportunity to manipulate the maladaptive neural activity underlying MS fatigue. The neuromodulatory potential of tES is widely shown on cognitive, perceptual, and motor processes (Yavari et al., 2018). As changes in brain activity were demonstrated in various neurological and psychiatric conditions, the clinical application of tES has been increasingly progressed with the aim to restore pathological brain function and to improve related symptoms (Sale et al., 2015).

In the following, we will first discuss evidence for the therapeutic potential of transcranial direct current stimulation (tDCS) on cognitive fatigue in people with MS. Moreover, we will emphasize the functional importance of altered neural oscillatory pattern in fatigue pathogenesis and discuss the possible advantage of transcranial alternating current stimulation (tACS) application for cognitive fatigue treatment. Table 3 presents an overview of studies evaluating tES effects on objective cognitive fatigability in people with MS and healthy controls.

**Table 3.** *Overview of studies evaluating transcranial electrical stimulation (tES) effects on objective cognitive fatigability.*

| Reference | Parameter | Sample Size | Stimulation Design | Study Design | Results |
|---|---|---|---|---|---|
| **tDCS Studies** | | | | | |
| Borragan et al. (2018) | RT, Accuracy | 20 HC | Position: left DLPFC<br>Parameters: 1.5 mA for 25 min<br>Average current density: 0.06 mA/cm² | Three blocks of PVT;<br>Between first and second block of PVT, participants performed cognitive demanding working memory task;<br>From the beginning to the second block, participants received anodal or sham tDCS (within-subject design) | Anodal tDCS compared to sham tDCS had no impact on behavioral performance decrements over time;<br>tDCS-related interhemispheric shift in cortical oxygenation after stimulation offset |
| Fiene et al. (2018) | RT, P300 amplitude, and latency | 15 MS (14 RR, 1 SP) | Position: left DLPFC<br>Parameters: 1.5 mA for circa 30 min<br>Average current density: 0.06 mA/cm² | Three blocks of SRT task and auditory oddball paradigm;<br>During second block, participants received anodal or sham tDCS (within-subject design) | Anodal tDCS compared to sham tDCS caused a decrease in RT and an increase in P300 amplitude, which persisted after the end of stimulation |
| Hanken et al. (2016) | RT, Accuracy | Study I: 52 HC<br>Study II: 46 MS (18 RR, 28 PP+SP) | Position: right parietal (Study I+II) or right frontal (Study I)<br>Parameters: 1.5 mA for 20 min<br>Average current density: 0.04 mA/cm² | Visual vigilance task for 40 min (Study I) or 20 min (Study II);<br>Anodal or sham tDCS for 20 min (between-subject design) | Anodal tDCS compared to sham tDCS counteracted the time-on-task RT decrements (in people with MS and healthy controls) |
| McIntire et al. (2014) | RT, Accuracy | 30 HC | Position: left DLPFC<br>Parameters: 2 mA for 30 min<br>Average current density: 0.199 mA/cm² | Five blocks of PVT every two hours after initial baseline assessment;<br>Anodal tDCS with placebo gum or sham tDCS with placebo or caffeine gum after 22 h of wakefulness (between-subject design) | Anodal tDCS compared to sham tDCS and caffeine gum prevented vigilance decrements over time and led to better subjective ratings of fatigue, drowsiness, and energy;<br>Positive effects lasted at least six hours |

**Table 3.** *Cont.*

| Reference | Parameter | Sample Size | Stimulation Design | Study Design | Results |
|---|---|---|---|---|---|
| McIntire et al. (2017) | RT, Accuracy | 50 HC | Position: left DLPFC<br>Parameters: 2 mA for 30 min<br>Average current density: 0.199 mA/cm² | Five blocks of PVT every two hours after initial baseline assessment;<br>Anodal tDCS with placebo gum or sham tDCS with placebo or caffeine gum early or late in the experiment (after 18 or 22 h of wakefulness) (between-subject design) | Anodal tDCS applied early in the experiment compared to sham tDCS led to improved attentional accuracy and RT lasting for six hours |
| Nelson et al. (2014) | RT, Accuracy | 19 HC | Position: left DLPFC<br>Parameters: 1 mA for 10 min<br>Average current density: 0.028 mA/cm² | Anodal, cathodal, or sham tDCS early (first 10 min) or late (last 10 min) during a 40 min vigilance task (within-subject design) | Especially early anodal and cathodal tDCS significantly improved task performance compared to sham tDCS |
| Sarasso et al. (2019) | Accuracy | 45 HC | Position: right or left PPC<br>Parameters: 1.5 mA for 15 min<br>Average current density: 0.06 mA/cm² | Two blocks of a visual vigilance task;<br>Between block participants received right-anodal-left-cathodal, right-cathodal-eft-anodal, or sham tDCS (between-subject design) | Right-cathodal-left-anodal tDCS counteracted the time-on-task decrease in performance accuracy compared to right-anodal-left-cathodal and sham tDCS |
| **tACS Studies** | | | | | |
| Loeffler et al. (2018) | RT, Accuracy | 24 HC | 40 Hz gamma tACS over visual cortex<br>Parameters: 1 mA for 30 min | tACS was applied during second block of a vigilance task (the first block taken as baseline) (between-subject design) | 40 Hz tACS significantly decreased the time-on-task related slowdown of RT compared to sham tACS |
| Clayton et al. (2019) | RT, Accuracy | 178 HC in four studies | 10 Hz alpha tACS over posterior cortex<br>Parameters: 2 mA for 11 min | Visual and auditory sustained attention task performance across four blocks;<br>10 Hz, 50 Hz, or sham tACS were applied during second and third block (within-subject design) | Alpha tACS compared to 50 Hz and sham tACS exerted a stabilizing effect on accuracy and RT and generally limited the slope of performance deteriorations or improvements over time (specific to visual domain) |

Abbreviations: *DLPFC,* dorsolateral prefrontal cortex; *HC,* healthy controls; *MS,* multiple sclerosis; *PP,* primary progredient MS form; *PVT,* Psychomotor Vigilance Task; *RT,* reaction time; *SP,* secondary progredient MS form; *SRT,* simple reaction time task; *tACS,* transcranial alternating current stimulation; *tDCS,* transcranial direct current stimulation

#### 3.1.6.1 Neuromodulation of the Fatigue Circuit by tDCS

tDCS is one of the most frequently used tES techniques that delivers a constant, low-intensity electrical current to the brain, resulting in modulation of cortical excitability (Nitsche et al., 2003). The current is steadily flowing between two or more surface electrodes (anode and cathode) placed on the scalp. The external electric field forces a shifting in intracellular ions in cortical pyramidal cells, thereby modifying internal charge and resting membrane potential. The stimulation-induced effects of current flow parallel to the somatodendritic axis in the target region depend on current polarity. Generally, anodal tDCS enhances cortical excitability via depolarization of resting membrane potentials, whereas cathodal tDCS decreases cortical reactivity via hyperpolarization of neuronal membranes (Nitsche & Paulus, 2000). Excitability-enhancing effects of anodal tDCS have been successfully demonstrated to outlast the stimulation period by several minutes to hours proposed to result from long-term synaptic changes in the stimulated region (Monte-Silva et al., 2013; Nitsche et al., 2002).

In healthy participants, tDCS over the left dorsolateral prefrontal cortex (DLPFC) has been shown to mitigate fatigue-induced decrements in vigilance performance over time (McIntire et al., 2014; McIntire et al., 2017; Nelson et al., 2014). McIntire et al. (2017) showed that tDCS was more beneficial than caffeine consumption in counteracting subjective state-fatigue and objective vigilance task decline during prolonged wakefulness. While these studies suggested tDCS as an effective fatigue countermeasure to maintain vigilance performance, one study failed to show effects of frontal tDCS on performance decline in a high cognitively demanding working memory task over time (Borragán et al., 2018). Besides stimulation of prefrontal brain regions, bilateral tDCS over the parietal cortex has further been shown to prevent fatigability in visual detection performance in healthy subjects (Sarasso et al., 2019).

For pathological MS-related fatigue, several studies have investigated the efficacy of tDCS over the fatigue circuit with the aim to restore altered neural excitability and improve subjective exhaustion. Positive effects of anodal tDCS over the left DLPFC (Ayache et al., 2016, Chalah, Riachi, et al., 2017b; Charvet et al., 2018; Saiote et al., 2014), the bilateral primary somatosensory cortex (Cancelli et al., 2018; Tecchio et al.,

2014; Tecchio et al., 2015), or the bilateral primary motor cortex (Ferrucci et al., 2014) were shown on subjective trait- and state-fatigue in people with MS. These studies gave important insight into the causal relevance of the targeted brain regions in fatigue pathogenesis. However, tDCS-induced improvements in MS-related objective cognitive fatigue parameters have rarely been the focus of investigation. Recently, Hanken, Bosse, et al. (2016) examined tDCS effects on fatigability measured as vigilance decrements with time-on-task in MS. Results demonstrated that anodal stimulation over the right parietal cortex as part of the vigilance network delivered for 20 min could counteract the reaction time increase during prolonged testing compared to sham. Yet, subjective state-fatigue increased independent of stimulation condition. Likewise, we investigated effects of tDCS on cognitive fatigue-associated behavioral and electrophysiological parameters in people with MS (Fiene et al., 2018). We showed that anodal tDCS of the left DLPFC for about 30 min caused an increase in P300 amplitude that persisted after the end of stimulation and reduced the fatigability-related increase in reaction time over the course of the testing session in comparison to sham. However, in line with the study by Hanken, Bosse, et al. (2016), stimulation did not counteract the increase in subjective state-fatigue with time-on-task. This dissociation between the feeling and the behavioral characteristics of fatigue might suggest that while a single session of anodal tDCS could lead to improvements in objective fatigability parameters, multiple repetitive sessions might be necessary to induce cumulative changes in the fatigue network that lead to subjectively perceivable changes in the feeling of fatigue (Ayache et al., 2017; Cancelli et al., 2018; Chalah, Lefaucheur, & Ayache, 2017; Charvet et al., 2018; Ferrucci et al., 2014; Saiote et al., 2014; Tecchio et al., 2014; Tecchio et al., 2015). Therefore, stimulation dosage and duration are presumably critical aspects that need to be considered for the development of effective stimulation protocols targeting subjective and objective fatigue symptoms.

#### 3.1.6.2 Role of Neural Oscillations in Cognitive Fatigue and its Modulation by tACS

Cognitive fatigue has not only been associated with altered neural excitability, previously targeted by tDCS but has also been related to alterations in neural oscillatory activity (Buyukturkoglu et al., 2017; Vecchio et al., 2017). In healthy subjects, a systematic shift from fast to low frequency waves has been reported during a reduced level of arousal

(Klimesch, 1999). Cognitive fatigability in healthy subjects has been repeatedly shown to be associated with power increase in the theta (4–8 Hz) and alpha (8–14 Hz) frequency band over frontal, central, and parietal brain regions with time spent on sustained attention tasks (Boksem et al., 2005; Craig et al., 2012; Klimesch, 1999; Shigihara et al., 2013; Wascher et al., 2014). Power increases were positively correlated with a decline in task performance (e.g., increased reaction time and error rates) as well as with subjective state-fatigue ratings (Boksem et al., 2005). Besides power changes, weakened fronto-parietal coupling in the alpha band, as well as increases in characteristic path length in the alpha and theta band, pointing to a less efficient information transfer, have been shown with cognitive fatigability (Dimitrakopoulos et al., 2018; Liu et al., 2010; Sun et al., 2014). Likewise, the examination of oscillatory patterns in people with MS showed an impaired connectivity balance in a parieto–occipito–temporal network in the alpha and beta band in patients with subjective trait-fatigue measured by total MFIS score (Vecchio et al., 2017). The level of subjective trait-fatigue in patients has further been shown to correlate positively with increased resting state functional connectivity between frontal regions in the theta and beta band as well as with an anterior–posterior increase in beta band connectivity (Buyukturkoglu et al., 2017). Thus, progressive power increases and connectivity distortions in low frequency bands have been interpreted as possible indices of cognitive fatigability. However, alpha and theta activity have also been assigned a positive functional role in maintaining an alert state. Theta power and theta band phase synchronization between medial and lateral prefrontal areas have been shown to increase following errors or negative feedback on sustained attention tasks (Cavanagh et al., 2009; Van de Vijver et al., 2011). This suggests a central role of theta band activity in cognitive control during prolonged cognitive testing. Furthermore, correlations between frontal activity and posterior alpha power may point to a modulatory role of theta-driven frontal activity on alpha oscillations in sensorimotor regions, thereby controlling activity of task-relevant and -irrelevant brain regions (Clayton et al., 2015; Mathewson et al., 2014). Thus, levels of alpha and theta activity might rather be interpreted as an indicator of increased effort to maintain an alert state (Clayton et al., 2015; Klimesch, 1999). Specifically, disturbed coupling in these frequency bands might be a crucial factor leading to fatigability-related performance declines.

Assuming that the reported low frequency oscillatory patterns play a mechanistic role in the pathogenesis of cognitive fatigue, the manipulation of abnormal oscillations by tACS might be a central aspect of effective fatigue treatment. tACS involves the application of rapidly alternating electrical currents to the scalp and is assumed to cause periodic shifts in membrane potential and an entrainment (i.e., temporal phase alignment) of neural activity to the externally applied current (Reato et al., 2013; Tavakoli & Yun, 2017). Although the direct assessment of neural tACS effects in humans is still complicated by electrical artifacts in concurrent neural recordings, findings on behavioral effects during stimulation and analyses of electrophysiological stimulation aftereffects provide good evidence for the efficacy of tACS to modulate oscillatory activity in a phase- and frequency-dependent manner (Fiene et al., 2019; Schwab et al., 2018; Vosskuhl et al., 2018). Lasting power and connectivity changes at the stimulation frequency have been interpreted as spike-timing-dependent plasticity effects as a consequence of synchronized activity during stimulation (Schwab et al., 2018; Zaehle et al., 2010). To our knowledge, research on MS-related fatigue has not made use of the neuromodulatory potential of tACS so far.

Findings on a disturbed connectivity pattern within the fatigue circuit, together with oscillatory changes in low-frequency bands with time-on-task in healthy subjects, motivate the possible application of various tACS montages. As frontal theta activity has been related to monitoring of cognitive processes, tACS applied at low frequencies might be used to increase frontal cognitive control and to counteract performance decline over time. Based on findings of interrelations between theta and alpha activity, tACS applied in the theta range might also improve regulation of sensorimotor alpha power. In a recent tACS study on cognitive fatigue in healthy subjects, Loeffler et al. (2018) applied tACS in the gamma range during a vigilance task with the aim to decrease inhibitory alpha power over task-relevant cortical regions via cross-frequency interactions. Results showed that gamma tACS counteracted the reaction time increase with time-on-task, yet, effects on occipital alpha power remained speculative due to missing EEG recordings. In a study by Clayton et al. (2019), alpha tACS applied to the parieto-occipital cortex during sustained attention tasks has been shown to have an overall stabilizing effect on performance level with time-on-task. This result might support the notion that increased alpha activity does not merely reflect a decrease in attention state. As synchronized, low-

frequency activity seems to play an important role in maintaining cognitive control, bifocal tACS applied in the theta range over frontal cortices or in the alpha range over frontal and parietal areas might be effective in counteracting disturbed coupling typically seen with increasing fatigue levels (Clayton et al., 2015; Vecchio et al., 2017).

Overall, fatigue relates to a complex brain state involving multiple brain regions within the fatigue circuit. The current literature suggests an important role of alterations in local excitability as well as oscillatory activity and connectivity inside the fatigue network that might result from demyelination and axonal degeneration in MS. Even if speculative, hyperactivity in the fatigue network might be related to cognitive control and an increased attentional effort to maintain an attentional state. However, with time-on-task, this overactivation might not be sufficient to compensate for processing inefficiencies and coupling alterations in other parts of the network. This assumption implies a central role of connectivity patterns in fatigue pathogenesis and could explain variability in the efficacy of tDCS to counteract fatigue symptoms. The complex nature of fatigue-related neural mechanisms might be more holistically treated by taking into consideration local excitability deficiencies as well as altered connectivity within the whole fatigue circuit. Therefore, the use of tACS complementary to tDCS might help to decode cognitive processes underlying cognitive fatigue. A combination of brain stimulation and neuroimaging techniques might be best suitable to test the effects of tES protocols on local and global activity changes inside the fatigue circuit. For the development of patient-tailored stimulation protocols, it is essential to further investigate the variability in responsiveness to tES application among people with MS. Different patterns of brain damage and anatomical differences in the tES target region might lead to variable stimulation efficiency. In previous tDCS studies on subjective fatigue in MS, tDCS effect size has not been found to correlate with demographic characteristics of patients like age, disease duration, or disability (Ferrucci et al., 2014; Saiote et al., 2014). Positive correlations have been reported for tDCS efficiency with lesion load in the left frontal cortex as well as with baseline fatigue levels (Cancelli et al., 2018; Charvet et al., 2018; Saiote et al., 2014). Interestingly, Ferrucci et al. (2014) reported that the subgroup of responders was significantly younger than non-responders. This result might suggest that therapeutic benefits of tES might require residual metabolic activity leaving more space for functional improvements (Thibaut et al., 2015).

### 3.1.7 Conclusion and Outlook

Fatigue is one of the most common symptoms encountered in people with MS and the main cause of early retirement. Thus, the development of reliable diagnostic instruments is of utmost clinical and social relevance. Recent investigations to complement the subjective nature of fatigue diagnostics by objective fatigue measures (i.e., simple reaction time or P300 ERP) are an important step to an integral diagnostic process and treatment evaluation. While the value of fatigability parameters has previously been critically discussed based on its inconsistent relation with subjective fatigue levels, we emphasize that objective manifestations of fatigue should not substitute subjective fatigue assessment but complement it in fatigue diagnostics. The MS fatigue construct is as complex as its underlying neural causes and should be diagnosed and treated in a holistic manner. Differentiating between individual aspects of the fatigue construct and a clear referencing to the taxonomy in scientific communication will help to provide clarity in further research on MS fatigue. In the absence of a common MS fatigue therapy, neuromodulation by tES provides a promising alternative treatment approach and additionally enables the causal investigation of underlying pathological mechanisms. Since tES methods are economic, easy to apply, and well tolerated, they allow for a large-scale use in clinical practice. Former evidence for improvements in fatigue symptoms by tES application encourages further investigation of effective and patient-tailored stimulation protocols.

# 3.2 Effects of repetitive twice-weekly transcranial direct current stimulations on fatigue and fatigability in people with multiple sclerosis

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## 3.2.1 Abstract

*Background:* Fatigue is associated with a dramatically decreased quality of life in people with multiple sclerosis (pwMS). It refers to a constant subjective feeling of exhaustion and a performance decline, known as fatigability. However, inconsistency and heterogeneity in defining and assessing fatigue have led to limited advances in understanding and treating MS-associated fatigue. Transcranial direct current stimulation (tDCS) has emerged as a promising, non-pharmaceutical treatment strategy for subjective fatigue. However, whether repetitive tDCS also have long-term effects on time-on-task performance has not yet been investigated.

*Methods:* This pseudorandomized, single-blinded, and sham-controlled study investigated tDCS effects on behavioral and electrophysiological parameters. PwMS received eight twice-weekly 30-minute tDCS sessions over the left dorsolateral prefrontal cortex. Fatigability was operationalized as time-on-task-related changes in reaction time variability and P300 amplitude. Additionally, subjective trait and state fatigue ratings were assessed.

*Results:* The results revealed a significant tDCS effect on subjective trait fatigue ratings that lasted at least four weeks after the stimulations. However, the ratings declined after both anodal and sham tDCS. No effects were found on subjective state fatigue and objective fatigability parameters. Linear Mixed Models and Bayesian Regression models likewise favored the absence of a tDCS effect on fatigability parameters.

*Conclusions:* The results confirm the complex relationship between MS-associated fatigue and fatigability. Reliable and clinically relevant parameters need to be established to extend the potential of tDCS for treating fatigability. Furthermore, our results indicate that consecutive stimulations rather than twice-weekly stimulations should be the preferred stimulation scheme in future studies.

## 3.2.2 Introduction

Fatigue is a challenging symptom of several neurological disorders. In contrast to tiredness, fatigue describes the overwhelming feeling of exhaustion, which manifests both cognitively and physically and does not resolve with rest or sleep. With up to 80% probability of occurrence, it is also one of the most common symptoms of multiple sclerosis (MS; Cook et al., 2013). MS is a primarily neuroinflammatory disease of the central nervous system with neurodegenerative features. Thus, symptoms vary according to the inflammatory lesion site. Fatigue, together with motor impairments, is considered the symptom that most reduces the quality of life in people with multiple sclerosis (pwMS; Yamout et al., 2013) and constitutes the leading cause of early retirement (Simmons et al., 2010).

Developing efficient therapeutic methods for overcoming fatigue is thus of high clinical relevance. However, there is no general agreement on which treatment method is most effective for treating MS-associated fatigue. A non-invasive, non-pharmaceutical method that has been of interest in recent years is transcranial direct current stimulation (tDCS). During tDCS, a constant low-intensity electrical current is applied via two or more surface electrodes on the scalp resulting in modulation of cortical excitability. This is generally described via a shift in resting membrane potential that leads to depolarization (anodal) or hyperpolarization (cathodal) of neuronal membranes (Nitsche et al., 2004; Nitsche & Paulus, 2000; Reed & Cohen Kadosh, 2018). While the described modulation of cortical excitability is reversible (Cohen Kadosh et al., 2012), other studies report long-term effects of tDCS via long-term potentiation (Monte-Silva et al., 2013; Nitsche et al., 2004). Furthermore, these tDCS effects seem to depend on several parameters, such as electrode-to-cortex distance and cerebrospinal fluid thickness, as well as the orientation of pyramidal neurons (Bikson et al., 2019). Due to fatigue-related functional abnormalities in the cortico-striato-thalamo-cortical network (Arm et al., 2019;

Palotai & Guttmann, 2020), several studies investigated the efficacy of tDCS in restoring altered neuronal excitability in pwMS. The majority of those studies stimulated the left dorsolateral prefrontal cortex (DLPFC) for at least three or more consecutive sessions (Ayache et al., 2016; Ayache et al., 2017; Chalah et al., 2020; Chalah, Lefaucheur, & Ayache, 2017; Chalah, Riachi, et al., 2017b; Charvet et al., 2018; Saiote et al., 2014) and reported positive effects on subjective fatigue ratings. Other studies also improved subjective fatigue by stimulating the bilateral primary somatosensory cortex (Cancelli et al., 2018; Tecchio et al., 2014; Tecchio et al., 2015) or the bilateral primary motor cortex (Ferrucci et al., 2014; Workman et al., 2020).

In recent years, however, there has been an increasing discussion that fatigue not only manifests in an ongoing, subjectively perceived exhaustion but is also associated with a higher level of exhaustion. This is supported by studies showing that the individual's cognitive performance, as well as the subjective feeling of momentary exhaustion, fluctuates significantly during the day, typically peeking in the late afternoon (Claros-Salinas et al., 2010; Powell et al., 2017). Additionally, Dettmers et al. (2021) recently reported that cognitive fatigability rather than subjective fatigue predicts the employment status of pwMS. Thus, cognitive fatigue is characterized by subjective and objective changes. While subjective fatigue can be subdivided into a trait (long-term) or state (momentary) component, objective fatigue is, per definition, state-dependent and refers to the failure to maintain one’s own individual optimal performance over time (Holtzer et al., 2011; Kluger et al., 2013). This fatigue-related performance decline, often referred to as fatigability, can be quantified as a change in performance with time-on-task. According to a model proposed by Hanken et al. (2014), subjective fatigue results from activated immune-to-brain pathways, innervating interoceptive and homeostatic brain areas, leading to the subjective feeling of fatigue. Furthermore, this results in increased interoceptive interference and distracts cognitive processes, manifesting in performance decline. However, performance decrements in alertness or vigilance tasks may also result from MS-induced focal brain atrophy, especially in frontal areas (Ayache & Chalah, 2017; Hanken et al., 2014). It is still an open question whether subjective fatigue and fatigability in pwMS are associated or independent from one another.

In healthy participants, anodal tDCS over the left DLPFC prevented vigilance decrements in sleep-deprived participants and improved subjective fatigue ratings

(McIntire et al., 2014; McIntire et al., 2017; Nelson et al., 2014). Likewise, healthy participants maintained and even improved their working memory performance in an hour-long two-back task while receiving anodal tDCS compared to sham stimulation (Karthikeyan et al., 2021). In pwMS, anodal stimulation led to decreased reaction times with time-on-task, but only in participants suffering from mild to moderate cognitive fatigue (Hanken, Bosse, et al., 2016). Fiene et al. (2018) repetitively assessed reaction times as well as P300 amplitudes and latencies while they applied either anodal or sham tDCS in pwMS. The authors report that anodal, compared to sham stimulation, counteracted fatigability-related cognitive exhaustion resulting in greater P300 amplitudes and a reduced increase in P300 latency and reaction times in pwMS. However, both studies reported that stimulation did not counteract the increase in subjective state fatigue ratings, despite the sustained or improved behavioral performance.

The previous literature has shown that a single session of tDCS can improve fatigability-related performance decrements in pwMS. In contrast, multiple repetitive tDCS sessions are necessary to induce subjectively perceivable changes in the global feeling of fatigue (Linnhoff et al., 2019). Accordingly, the current study investigates the effects of repetitive twice-weekly anodal tDCS sessions on objective fatigability development as well as subjective state fatigue ratings in pwMS. To our knowledge, this is the first study to explore tDCS effects on fatigability after repetitive stimulations in pwMS. We hypothesized that tDCS would reduce subjective fatigue ratings and the fatigability-related performance decline with time-on-task.

## 3.2.3 Methods

### 3.2.3.1 Participants

We enrolled 18 participants (male = 3) aged 23 to 65 years in this study. All participants were diagnosed with clinically definite MS according to the McDonald criteria and were native German speakers. All participants had relapsing-remitting MS. Baseline group characteristics are listed in Table 4.

**Table 4.** *Baseline group characteristics.*

| | **anodal group (*n* = 9) mean (± *SD*)** | **sham group (*n* = 9) mean (± *SD*)** | |
|---|---|---|---|
| gender f/m | 8/1 | 7/2 | |
| age [years] | 40.44 (14.37) | 40.89 (10.49) | $p = .825$ |
| Disease duration [years] | 5.22 (4.55) | 8.44 (8.81) | $p = .350$ |
| EDSS [points] | 3.00 (1.79) | 2.78 (1.66) | $p = .718$ |
| BDI-FS [points] | 2.33 (0.71) | 2.33 (2.69) | $p = .339$ |
| $WEIMuS_{tot}$ [points] | 33.89 (10.37) | 44.44 (9.79) | $p = .093$ |
| $WEIMuS_{cog}$ [points] | 18.44 (5.05) | 23.00 (4.47) | $p = .092$ |
| $WEIMuS_{phy}$ [points] | 15.44 (6.15) | 21.44 (6.29) | $p = .132$ |
| cross-over participation | 4 | 5 | |

*BDI-FS,* Becks Depression Inventory Fast Screen; *EDSS,* Expanded Disability Status Scale; *WEIMuS,* Wuerzburg Fatigue Inventory for Multiple Sclerosis

Inclusion criteria were a minimum of three months since the last relapse or use of corticosteroids, no paresis of the upper limb, no previous or current neurological or psychiatric comorbidities, and no treatment with fatigue medication. Participants neither had a diagnosed depression nor pharmacological treatment with antidepressants. The disease-modifying MS therapy consisted of glatiramer acetate ($n$ = 5), interferon-beta ($n$ = 3), fingolimod ($n$ = 3), teriflunomide ($n$ = 2), and dimethyl fumarate ($n$ = 1). Four participants had no MS medication. Additionally, participants hat to meet tDCS criteria such as no cardiac arrhythmias or pacemaker, no pregnancy, no metal in the cranium except in the mouth (retainer), no surgical clips in or near the brain, no epilepsy, or epileptic seizures in the lifetime, no recurring unexplained blackouts, and no chronic skin diseases on the shoulder, face, and scalp. All participants reported having normal hearing and normal or corrected-to-normal vision. They were recruited from the outpatient pool of the University Hospital of Magdeburg and received monetary reward (Euro 80 in total) for participation in the study. The study was approved by the local ethics committee of the University Clinic of Magdeburg and was conducted in accordance with the Declaration of Helsinki. All participants gave written informed consent before participation.

### 3.2.3.2 Procedure

We designed a placebo-controlled study in two phases to assess the efficacy of anodal tDCS over the left DLPFC. In "Phase I", we used a between-subject design and pseudorandomly allocated participants to an anodal (n = 9) or sham tDCS group (n = 9). After completing the first phase, nine participants (four from anodal, five from sham group) agreed to participate in "Phase II", in which they crossed groups and participated a second time after a 12 weeks wash-out interval.

Each phase consisted of three experimental sessions in which subjective fatigue and fatigability scores were assessed. In between pre- and post-session, eight anodal stimulation sessions were administered. A follow-up session took place four weeks after the post-session. The study design is illustrated in Figure 5.

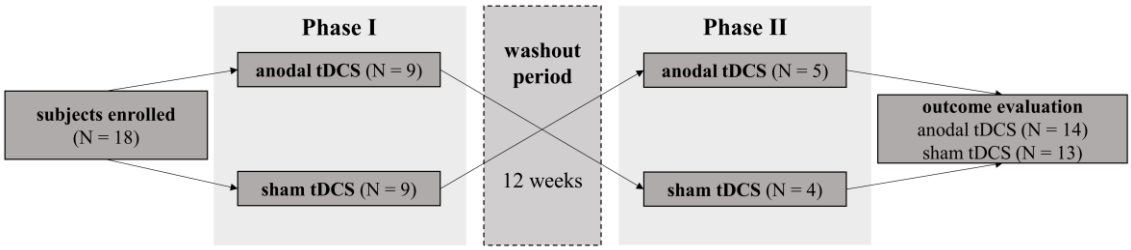


**Figure 5.** Schematic design of the study. A two-phased, randomized controlled, cross-over study with two groups (anodal and sham tDCS, transcranial direct current stimulation).

Initially, participants signed informed consent and completed several questionnaires to assess their disease history and their ability to participate in the experiment (tDCS questionnaire). At the beginning of each session, participants rated their current mood (BDI-II) and subjective trait fatigue (WEIMuS, Flachenecker et al., 2006). The WEIMuS consists of 17 items on a five-point Likert scale from "almost never" to "almost always" and evaluates a total score, as well as individual scores for the physical and cognitive fatigue dimensions. Higher scores reflect a stronger fatigue expression. Afterward electroencephalogram (EEG) was prepared, and the EEG session started. Each

experimental session (pre, post, and follow-up session) consisted of three test blocks. Each test block (B1, B2, and B3) consisted of three tasks: a serial reaction time task (SRT), an auditory oddball task (see below), and a 10-point numerical rating scale (NRS), where participants rated their current feelings of fatigue depending on "how mentally exhausted" they felt at the time, from 0 (not at all) to 10 (extremely exhausted). Test blocks were repeated three times in order to assess time-on-task changes relative to the respective individual and day-dependent baseline value (performance at B1). All three experimental sessions took place at the same time of day. The experimental design of each phase is illustrated in Figure 6.

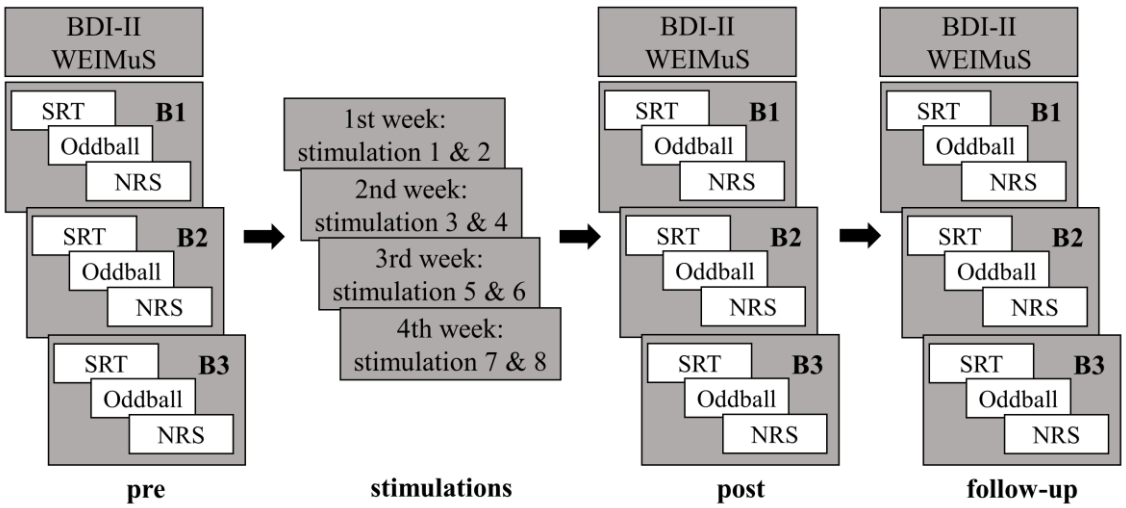


**Figure 6.** Experimental procedure. The experiment consisted of three experimental sessions, with three test blocks each (B1, B2, and B3). In each test block, fatigability and subjective state fatigue scores were assessed. RT variability was evaluated via a serial reaction time task (SRT), P300 during an auditory oddball task, and subjective state fatigue via a 10-point numerical rating scale (NRS). Additionally, depression scores using the Becks Depression Inventory (BDI-II) as well as subjective trait fatigue scores using the Wuerzburger Fatigue Inventory (WEIMuS) were assessed. In between pre- and post-session, eight anodal or sham stimulation sessions were administered.

#### 3.2.3.3 Experimental tasks

Behavioral performance was evaluated using the SRT task adapted from Woods et al. (2015). Thus, participants were asked to press the space bar with their dominant hand as quickly as possible when a target in the form of a bulls-eye stimulus was presented on the computer screen. One test block consisted of 120 stimuli and lasted approximately 4.5 min. The interstimulus intervals ranged from 1000 to 2000 ms in 250 ms steps, and they were pseudorandomly used with equal probability. The stimulus presentation lasted 200 ms. The bulls-eye had a diameter of 5.72° of visual angle and was presented in black color on a white screen. Between stimuli, a fixation cross was shown.

To evoke the P300, we utilized an acoustic oddball paradigm. Therefore, a randomized series of frequent standard tones (1000 Hz, 80 %) and deviant "target" tones (2000 Hz, 20 %) were presented. Both stimuli had a presentation time of 70 ms (10 ms rise/fall time) and were presented binaurally via headphones [Sennheiser HD 65 TV] at 70 dB. The interstimulus interval varied between 1500 ms and 2000 ms. During the task, participants were instructed to press the space bar when the target tone was presented and to ignore other stimuli. One test block consisted of 240 standard and 60 deviant tones and lasted approximately 10 minutes with a one-minute break. Both tasks were presented using the Presentation software [Neurobehavioral Systems Inc, USA].

#### 3.2.3.4 tDCS design

The anodal tDCS group received eight sessions of active treatment, while the sham tDCS group received placebo treatment. The stimulations were administered two times a week for four weeks, and at least one day of rest was required between sessions. During the stimulation, participants were at rest. A battery-driven DC stimulator [DC Stimulator Plus, NeuroConn, Germany] delivered the stimulation using two rubber electrodes covered with saline-soaked sponges. The anode (5×7 cm) was placed over the left DLPFC (F3 according to the international 10-20 system for EEG electrode placement). The reference electrode (5×10 cm) was placed extracephalically over the right shoulder to prevent unwanted cephalic polarization effects under the return electrode (Nasseri et al., 2015). For the anodal tDCS condition, a direct current with an intensity of 1.5 mA was applied for 30 min stimulation with a 15 s fade in/out time. For the sham tDCS condition, we used the 15 s fade in – 30 s stimulation – 15 s fade out approach suggested by Ambrus

et al. (2012) to simulate skin sensations and ensure blinding. The impedance of stimulation electrodes was kept below 10 kΩ.

At the end of every stimulation session, participants were asked to fill out a short questionnaire on tDCS side effects. The questionnaire was designed according to the consensus tDCS guidelines (Antal et al., 2017) and asked whether and to what extent participants felt any of the following sensations due to stimulation: headache, nausea, dizziness, loss of concentration, fatigue, metallic taste, skin irritation or itch, tickle or heat on the scalp. The numeric rating scale ranged from 0 = no sensation to 3 = strong sensation.

#### 3.2.3.5 EEG signal recording and preprocessing

EEG was recorded at Cz, Pz, POz, P3, PO3, P4, and PO4 using Ag/AgCl-electrodes mounted in an elastic cap [EasyCap GmbH, Germany]. The ground electrode was attached to the AFz position, and all channels were referenced to the nasion. The electrooculogram (EOG) was recorded using two electrodes placed below the pupil (vertical EOG) and to the external canthus of the left eye (horizontal EOG). The data was recorded by Brain DC amplifier [Brain Products, Germany] and the corresponding software [BrainVision Recorder, Version 1.20, Brain Products, Germany] sampled at 1000 Hz. Impedances were kept below 5 kΩ. EEG preprocessing and data analysis were carried out in BrainVision Analyzer [Version 2.2.2, Brain Products GmbH, Gilching, Germany]. The EEG data were off-line band-pass filtered from 0.1 to 30 Hz and corrected for eye-movement artifacts using the Gratton and Coles method (Gratton et al., 1983). Using automatic artifact rejection, epochs with amplitudes exceeding ± 75 µV, voltage steps greater than 100 µV, or an absolute difference of 200 µV between the minimum and maximum voltage within 200 ms intervals were excluded. For P300 analysis, trials in which participants gave the correct answer were segmented into epochs from -200 ms to 800 ms relative to stimulus onset. The 200 ms pre-stimulus interval served as a baseline. Averages for the standard and deviant tones were computed separately for each block, session, and condition. The final peak detection was performed on single-subject deviant waves, and the P300 was quantified as the mean amplitude between 250 and 450 ms at electrode Pz.

#### 3.2.3.6 Statistical analysis

To explore blinding success, we analyzed the tDCS questionnaires by summing up all ratings of the eight stimulation sessions and checking for group differences using the Mann-Whitney U test (Phase 1) or Wilcoxon-signed-ranked test (Phase II).

All data were analyzed using linear mixed models (LMMs). This way, we accounted for the unbalanced study design as well as the non-independence of the data. We used R Statistical Software (version 4.2.0, R Core Team, 2022) for statistical analyses and production of all plots. LMMs were performed using the lmer function from the *afex* package (Singmann et al., 2022a). P values were obtained using Sattersthwaite´s approximation method. For subjective trait fatigue scores, we used the items of the cognitive dimension of the WEIMuS questionnaire ($WEIMuS_{cog}$). To assess objective fatigability, we used reaction time variability (RT variability) and P300 peak amplitudes. Subjective state fatigue was evaluated via the NRS ratings. We computed delta scores for the three time-on-task parameters by subtracting scores of block B3 from baseline responses in block B1. This improved the model fit and met the fatigability definition from Kluger et al. (2013), according to which fatigability is defined as a performance decline with time-on-task. RT variability was analyzed using the standard deviation of reaction times. Subjective data, RT variability, and P300 amplitudes were considered as dependent variables. *Session* (pre, post, fu) and *group* (anodal, sham), as well as their interaction, were considered as fixed factors. Data from the sham group at pre session were used as baseline. Subjects were used as random effects, thus accounting for the individual specific characteristics and the dual participation of some subjects resulting from the cross-over design. Furthermore, to account for order effects, we also included *order* as a covariate and compared both models using the Akaike Information Criterion (AIC). However, *order* as an additional factor did not improve any of the models. Thus, it was not included in the final model. We further used Bayesian Linear Regression Models using the *brms* package (Buerkner, 2017) with the same model formula and default priors to further evaluate our results when LMMs yielded no effect of tDCS on the fatigability parameters.

## 3.2.4 Results

### 3.2.4.1 tDCS adverse effects and blinding

All participants tolerated the stimulation well. No participant reported severe side effects or pain under the electrodes. In Phase 1, there were significant differences between anodal and sham group concerning tingling (Mann-Whitney U = 9.50, $p$ = .006), skin redness (Mann-Whitney U = 9.00, $p$ = .004), and itching under the electrode (Mann-Whitney U = 17.50, $p$ = .040). In all cases, this resulted from stronger feelings of the respective symptom in the anodal compared to the sham group. However, no participant reported having been aware of the stimulus condition when being asked at the end of the study. In Phase II, there are significant differences between the conditions concerning tingling (Wilcoxon signed-rank W = 26.50, $p$ = .042) and skin redness under the electrode (Wilcoxon signed-rank W = 21.00, $p$ = .036). Again, this resulted from higher ratings after anodal compared to sham stimulations. Finally, in Phase II, we asked participants when they thought they had received anodal and when sham stimulation. Two (2/9) participants guessed the order right, two (2/9) were not sure, and five (5/9) thought they received stimulations in the opposite order.

### 3.2.4.2 Subjective fatigue

*Subjective trait fatigue*

Changes in subjective trait fatigue ratings ($WEIMuS_{cog}$) are shown in Figure 7A. The model to predict the subjective cognitive fatigue ratings showed a significant effect of *session* [$F(2 , 57.12) = 26.824$, $p < .001$, $\eta_p^2 = .48$] but no significant effect of *group* [$F(1 , 61.20) = 0.089$, $p = .767$] and no significant interaction of *session* and *group* [$F(2 , 57.12) = 1.186$, $p = .313$]. Thus, cognitive fatigue scores generally decreased after repetitive stimulations. The initial ratings of trait fatigue were 20.64 points [$\beta_{intercept}$, 95% CI (16.96, 24.30)] in the sham group and 19.30 points ($\beta_{intercept} + \beta_{group}$) in the anodal group. After stimulations, fatigue was reduced by 7.69 points in the sham group [$\beta_{session}$, 95% CI (-10.20, -5.19)] and by 5.07 points in the anodal group ($\beta_{session} + \beta_{session*group}$). Bonferroni-corrected post hoc comparisons revealed significant reductions in fatigue scores from pre to post session [$t(57) = 7.005$, $p < .001$] and from pre to follow-up session [$t(57) = 5.324$, $p < .001$]. There were no further reductions from post to follow-up session

[$t(57) = -1.604$, $p = .343$]. However, reduced fatigue scores remained stable in both groups.

*Subjective state fatigue*

The subjective state fatigue ratings as a function of time-on-task separate for both groups and the three sessions are shown in Figure 7B. They increased by about one point during the pre-session in both groups (sham: 1.08 ± 1.26; anodal: 0.93 ± 1.07). In contrast, during the post session, the ratings increased by about 0.77 points (± 1.17) in the sham group and by only 0.57 points (± 1.28) in the anodal group. Similar results were found during the follow-up session (sham: 1.08 ± 1.26; anodal: 0.67 ± 1.36). However, the LMM to predict subjective state fatigue changes showed no significant main effects of *session* [$F(2 , 55.92) = 0.805$, $p = .452$] nor of *group* [$F(1 , 68.98) = 0.275$, $p = .602$] and no significant interaction [$F(2 , 55.92) = 0.089$, $p = .915$]. Consequently, the repetitive anodal stimulation appeared not to have affected the subjective fatigue increase during the performance of an exhaustive task. To further explore this, we additionally subjected the data to a Bayesian regression model using the same model formula.

The Bayesian model revealed that the interaction of *session* (pre, post) and *group* (sham, anodal) has a probability of only 52.28 % of being negative [Median = - 0.03, 95% CI (-1.13, 1.05)], which would indicate a reduced increase of subjective state fatigue in the anodal group during the post session compared to the sham group and pre session. Additionally, this interaction's significance is considered highly uncertain (18.61 % in ROPE). Thus, this further supports the evidence favoring an absent tDCS effect on subjective state fatigue increases with time-on-task.

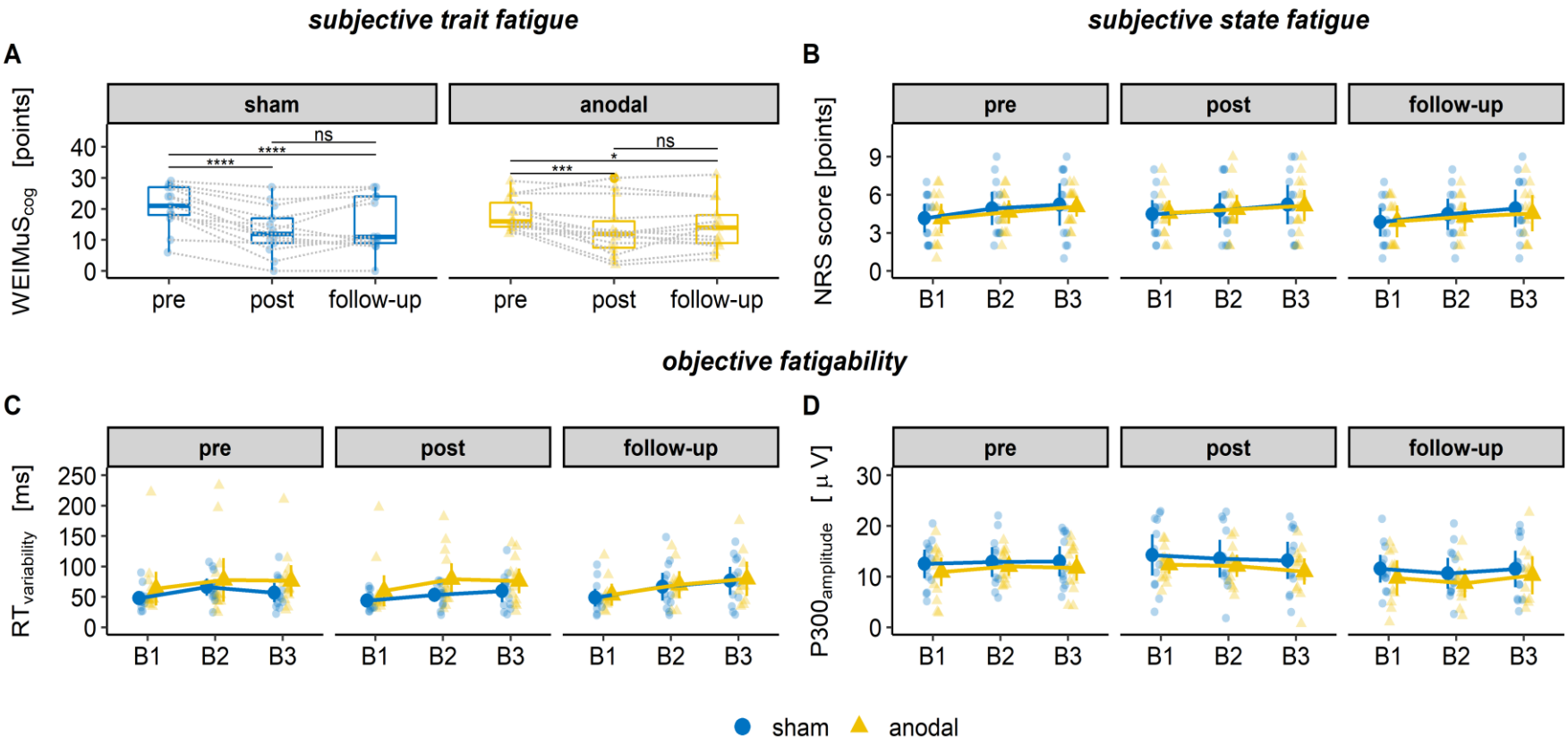


**Figure 7.** tDCS effects on fatigue and fatigability parameters. A: WEIMuS cognitive scores as a function of session (pre, post, follow-up) separate for sham and anodal group. B-D: Changes in numerical rating scores (NRS, B), reaction time variability (RT variability, C), and P300 amplitudes (D) as a function of block (B1, B2, and B3) (mean ± 95 % CI).

### 3.2.4.3 Objective fatigabilty parameters

*Reaction time variability*

RT variability similarly increased in both groups during all three sessions. Mean delta scores during pre session are 9.28 ms (± 13.16 ms) in the sham group and 12.98 ms (± 15.97 ms) in the anodal group. During the post session, RT variability increased by 15.92 ms (± 21.04 ms) in the sham group and by 16.54 ms (± 34.29 ms) in the anodal group. In contrast, during the follow-up, RT variability increased by 27.79 ms (± 31.50 ms) in the sham group and 26.18 ms (± 43.48 ms) in the anodal group. Consequently, the LMM to predict changes in RT variability with session and group showed no significant results [*session*: $F(2 , 55.92) = 2.645$, $p = .080$; *group*: $F(1 , 71.05) = 0.130$, $p = .720$; *session* x *group*: $F(2 , 55.92) = 0.076$, $p = .927$]. We again performed a Bayesian regression model to evaluate the results further. The interaction of *session* (pre, post) and *group* (sham, anodal) has a probability of only 60.42% of being negative [Median = - 3.24, 95% CI (-30.24, 23.61)], which would indicate that the RT variability increase during the post session is reduced in the anodal group compared to the sham group and the pre session. This interaction's significance is considered highly uncertain (16.53 % in ROPE). Consequently, an absent tDCS effect on RT variability increase during an exhaustive task is more likely. Figure 7C depicts the RT variability as a function of time-on-task separate for both groups and the three sessions.

*P300 peak amplitude*

P300 peak amplitudes increased during the pre session in the sham group (0.53 µV ± 2.86 µV) and the anodal group (0.81 µV ± 3.65 µV). Contrary, during the post session, they decreased in both groups (sham: -1.09 µV ± 4.60 µV; anodal: -1.37 µV ± 3.11 µV). During the follow-up, peak amplitudes decreased in the sham group (-0.05 µV ± 3.76 µV), while they increased in the anodal group (0.55 µV ± 5.01 µV). The LMM to predict the change in P300 peak amplitude with session and group showed no significant main effects [*session*: $F(2 , 55.62) = 2.601$, $p = .083$; *group*: $F(1 , 69.98) = 0.078$, $p = .781$] and no significant interaction [$F(2 , 55.62) = 0.154$, $p = .857$]. The Bayesian regression model revealed that the interaction of *session* (pre, post) and *group* (sham, anodal) has a probability of only 63.70 % of being negative [Median = - 0.63, 95% CI (-4.17, 2.89)] and the significance of this interaction is considered as highly

uncertain (16.89 % in ROPE). Consequently, as in the other fatigability parameters, this supports the conclusion that twice-weekly repetitive tDCS sessions did not affect P300 peak amplitude changes with time-on-task. P300 peak amplitude values as a function of time-on-task separate for both groups and the three sessions are shown in Figure 7D.

## 3.2.5 Discussion

This study investigated the effects of multiple, twice-weekly tDCS sessions on both subjective fatigue and objective fatigability parameters in people with MS-associated fatigue. Subjective trait fatigue ratings decreased significantly after both anodal and sham tDCS. However, we did not observe tDCS-specific effects on subjective state fatigue ratings or objective fatigability parameters.

### 3.2.5.1 Placebo effect

Our observed improvement of subjective trait fatigue after anodal stimulations is in line with a series of previous studies (Ayache et al., 2016; Ayache et al., 2017; Cancelli et al., 2018; Chalah et al., 2020; Chalah, Lefaucheur, & Ayache, 2017; Chalah, Riachi, et al., 2017; Charvet et al., 2018; Ferrucci et al., 2014; Tecchio et al., 2014; Tecchio et al., 2015; Workman et al., 2020). However, our study showed an improvement in subjective trait fatigue independent of the stimulation condition. This effect of sham stimulation might be related to a marked placebo effect. While this has not been systematically investigated in most previous tDCS studies, at least one study reported similar placebo effects (Saiote et al., 2014). In their study, the DLPFC was stimulated for 20 minutes on five consecutive days. Similar to our results, both the sham and the anodal condition significantly reduced fatigue ratings. According to the authors, methodological problems with self-report instruments may have contributed to the pronounced placebo effects during the sham condition and, in consequence, to a partial masking of the anodal tDCS effect. However, we used the WEIMuS questionnaire, which has been validated in a large MS sample and discriminates successfully between MS patients with and without fatigue. Additionally, it considered a time window of two weeks and was, therefore, suitable for our experimental design.

However, it is noteworthy that the repetitive stimulation design required intense caregiving over a significant amount of time, including conversations and

symptom exchange. This likely resulted in a stimulation-independent, positive interaction between the experimenter and the participants that, in turn, had a significant impact on the participants' subjective perception, further promoting the observed placebo effect.

#### 3.2.5.2 Objective fatigability parameters

We decided to use RT variability and P300 amplitude as objective fatigability parameters because they are easy and reliably assessed. Especially the P300 ERP components are widely used as an index of cognitive functioning. In pwMS, previous studies already demonstrated longer P300 latencies and smaller amplitudes associated with subjective trait fatigue (Chinnadurai et al., 2016; Fiene et al., 2018; Pokryszko-Dragan et al., 2016). Additionally, tDCS counteracted those fatigue-induced changes in pwMS (Fiene et al., 2018) and other clinical cohorts (Khedr et al., 2014; Nakamura-Palacios et al., 2012). On the other hand, higher subjective state fatigue ratings in healthy participants did not affect cognitive performance and had no impact on P300 components (Takács et al., 2019). Up to now, there is no consensus concerning clinically relevant and reliable outcome measurements for fatigability in MS, despite a wide variety of studies addressing this research question. Other electrophysiological parameters, such as sensory and sensorimotor gating or spectral power changes in the alpha and theta band, may be more suitable (Linnhoff et al., 2021; van der Linden et al., 2006; Wascher et al., 2014).

Furthermore, we investigated the parameters over a prolonged period of three blocks and assessed changes compared to baseline values. We assumed that subjective fatigue ratings and RT variability would increase with time-on-task while P300 amplitudes would decrease, as shown previously (Claros-Salinas et al., 2010; Fiene et al., 2018). However, in the present study, we did not observe robust results but instead revealed highly heterogeneous data patterns that differed inter- and intraindividual. Thus, while all participants reported an increase in subjective exhaustion, indicating that the chosen tasks effectively induced cognitive fatigability, they were mostly able to uphold their cognitive performance. A better approach to study fatigability development in the future might be to measure the changes in performance during sustained mental effort. Hence, to compare performance at the beginning and the end of an ongoing cognitively demanding task. Behavioral changes associated with an increase in subjective exhaustion have repeatedly been observed in studies using this approach (Cehelyk et al., 2019;

Gossmann et al., 2014; Hanken, Bosse, et al., 2016). Nevertheless, the literature remains heterogeneous regarding the existence of valid and reliable objective fatigability parameters (Linnhoff et al., 2019).

#### 3.2.5.3 Stimulation design

The stimulation design of the present study differed from previous investigations. We administered the stimulations offline and twice-weekly for four weeks, rather than in a single online session (Fiene et al., 2018; Hanken, Bosse, et al., 2016) or on consecutive sessions (Ayache et al., 2016; Cancelli et al., 2018; Chalah et al., 2020; Chalah, Riachi, et al., 2017b; Charvet et al., 2018; Saiote et al., 2014; Tecchio et al., 2015; Tecchio et al., 2014; Workman et al., 2020). Accordingly, our observations could be partly attributed to our non-daily stimulation design. Alonzo et al. (2012) examined the influence of daily vs. second daily stimulations on motor evoked potentials. They reported that daily tDCS sessions lead to a more significant increase in neuronal excitability than second daily tDCS. However, as a result of their physical impairment and chronic fatigue, pwMS often refrain from participating in time-consuming study protocols. Hence, when planning the study design, attention was paid to the study's external validity and practicability. In addition, Mortezanejad et al. (2020) treated pwMS with six second-daily stimulations. They found a significant reduction in fatigue ratings and an increase in quality of life while there were no effects after sham stimulation. Likewise, To et al. (2017) used a stimulation design similar to ours. They applied eight anodal stimulations over the left DLPFC for 20 minutes and reported positive effects on subjective fatigue ratings in people with fibromyalgia.

Moreover, we assessed all performance parameters before and after the offline stimulations, in which the participants were at rest, sitting in a comfortable chair in a quiet room. Contrary, other studies evaluated tDCS-related changes during or immediately following a single stimulation (Fiene et al., 2018; Hanken, Bosse, et al., 2016). Especially concerning the effects of tDCS on fatigability, this might have led to a crucial disadvantage in this study. Thus, Dedoncker et al. (2016) systematically reviewed tDCS studies over the DLPFC and reported that online task performance while the stimulation is applied results in greater performance improvements compared to offline task performance. However, the studies included in this meta-analysis only applied

stimulations during a single session. The lack of offline advantages might have been compensated by repetitive stimulation and its cumulative effects (Ayache et al., 2017; Chalah, Lefaucheur, & Ayache, 2017; Charvet et al., 2018). Additionally, for a clinical application, it is imperative to achieve long-term effects. Single tDCS sessions while performing a cognitively demanding task lose external validity and may only provide temporary improvements. Stimulations studies combined with cognitive training during the stimulations sessions might lead to cumulative effects and long-term fatigability improvements in pwMS (Charvet et al., 2018; Mattioli et al., 2016). However, this has yet to be systematically evaluated in future studies focusing on fatigability improvement in pwMS.

#### 3.2.5.4 Limitations and Perspectives

This study has several limitations. First, an interpretation of the results is limited by the small sample size and heterogeneous MS cohort – despite the careful inclusion of pwMS with similar disability status. Even though the majority of former studies on that topic used similar sample sizes, the results of our study cannot be generalized beyond the sample and must instead be considered as preliminary data. Furthermore, we decided to limit exclusion criteria to a minimum. While this improved the external validity, it may have prohibited the emergence of any positive effect of tDCS on fatigability. Thus, we included pwMS with elevated BDI values as long as they did not have a diagnosed depression or took antidepressants. Notably, there were only two participants with higher scores than the cutoff values, and these individuals were designated, by chance, to each of both groups. Therefore, it is unlikely that this influenced our results.

Second, the study is limited by the lack of functional or structural neuroimaging data. In recent years, an increasing number of studies have emerged showing interindividual variability of tDCS effects, limiting the efficacy of brain stimulation (Laakso et al., 2015; Mosayebi-Samani et al., 2021; Wiethoff et al., 2014). Mosayebi-Samani et al. (2021) investigated the association between individual anatomical parameters and tDCS-induced electric fields and explored which parameters predicted the physiological outcome. The authors found an association between electrical field values and cerebrospinal fluid thickness and electrode-to-cortex distance, and the parameters predicted physical outcomes. In addition, lesion load predicts tDCS effects on fatigue in pwMS (Saiote et al., 2014). Ideally, future studies should pay more attention to

these interindividual anatomical and clinical factors and focus more on personalized electrodes (Cancelli et al., 2018; Tecchio et al., 2014; Tecchio et al., 2015).

Finally, we included participants with at least a minimum degree of subjective trait fatigue. A more appropriate approach might have been to include participants according to their degree of fatigability. Thus, assuming that in pwMS, subjective fatigue and fatigability can jointly appear but also exist independent of one another (Hanken et al., 2014; Linnhoff et al., 2019), we cannot be sure that our sample initially suffered from fatigability. Future studies should pay more attention to that and explicitly investigate tDCS effects on participants with predetermined fatigability. Unfortunately, our sample size was too small to distinguish between high and low fatigability participants. However, reliable and valid objective parameters are required for this to be successful. And those have yet to be established.

#### 3.2.5.5 Conclusion

In this study, we investigated the effects of repetitive twice-weekly tDCS sessions on fatigue and fatigability in pwMS. Our results show a positive effect on subjective trait fatigue scores that lasted at least four weeks after the stimulations. However, this effect was independent of the stimulation scheme. No effects were observed on fatigability with time-on-task or subjective state fatigue scores. To this date, there is no consensus about the relationship between subjective trait fatigue and objectively measurable fatigability. Our study once again demonstrates the complex relationship between MS-associated fatigue and fatigability. Improving subjective fatigue should continue to be the focus in daily clinical practice. However, especially in the context of the increasing number of people with fatigue, as currently observed by *Long Covid* (Nalbandian et al., 2021), it is imperative to extend the current subjective fatigue diagnosis with objective parameters for a more holistic approach and to broaden its acceptance. Considering that there is no effective treatment of MS-related fatigue available and that tDCS is easy to apply and well-tolerated, even the demonstration of a low degree of fatigue relief in a minority of patients will substantially improve healthcare in pwMS suffering from fatigue. However, future studies should prefer repetitive stimulations session on consecutive days instead of a twice-weekly stimulation scheme.

# 3.3 Objective electrophysiological fatigability markers and their modulation through tDCS

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## 3.3.1 Abstract

*Objective:* Cognitive fatigability is a frequent symptom after sustained performance. Fatigability is evident in healthy subjects but is also often comorbid in several neuropsychiatric diseases. However, to date, clinical diagnostic almost solely relies on the self-reported subjective experience of fatigue. The goals of this present study were i) to complement the purely subjective fatigue diagnostic with objective electrophysiological fatigability parameters and ii) to prove the potential therapeutic application of transcranial direct current stimulation (tDCS) as a fatigability intervention.

*Methods:* We performed a pseudo-randomized, sham-controlled, parallel-group trial. Forty healthy participants received either anodal or sham tDCS over the left dorsolateral prefrontal cortex (DLPFC) while they performed an exhaustive cognitive task to induce cognitive fatigability. To assess fatigability changes, we analyzed variations of prepulse inhibition (PPI) and P50 suppression as well as frontomedial theta and occipital alpha power with time-on-task.

*Results:* The task reliably induced subjective exhaustion in all participants. Furthermore, we confirmed fatigability-related increases in frontomedial theta and occipital alpha power throughout the task. Additionally, fatigability significantly reduced PPI as well as P50 sensory gating. Anodal tDCS over the left DLPFC successfully counteracted fatigability and reduced the fatigability-related increase in alpha power as well as the decline in both gating parameters.

*Conclusion:* Occipital alpha and sensorimotor/sensory gating are suitable parameters to assess the severity of fatigability objectively. Anodal tDCS can counteract fatigability and has therapeutic potential for the treatment of fatigability in neuropsychiatric diseases.

*Significance:* Fatigability can be objectively assessed by electrophysiological measures and attenuated by tDCS.

## 3.3.2 Introduction

Fatigue is a complex and multilayered construct leading to an overall feeling of exhaustion, loss of motivation, and behavioral performance decrements (Boksem & Tops, 2008). It is a significant cause for traffic accidents (Philip, 2005) or accidents in other work-related environments (Caldwell et al., 2019). In addition, fatigue is often comorbid to a wide range of psychological and somatic disorders, e.g., depression, Parkinson's disease, cancer, and multiple sclerosis (MS). Among patients with MS, fatigue affects up to 75 % of patients (Fisk et al., 1994) and is the main reason for early retirement (Simmons et al., 2010).

However, even with fatigue having such a significant impact, the pathogenesis and concept of fatigue are poorly understood. One important approach to a better understanding of fatigue is to use a unified taxonomy. A recent review (Linnhoff et al., 2019) summarized all existing terms in a coherent scheme. Hence, fatigue can be subdivided into psychosocial, physical, and cognitive fatigue, the latter being a result of cognitive exhaustion. Cognitive fatigue can further be subdivided into subjective and objective fatigue. While subjective fatigue either describes the ongoing (trait) or momentary (state) perceived feeling of exhaustion, objective fatigue refers to a measurable performance decline during the execution of a cognitively demanding task. Thus, for further research, it is of utmost importance to discriminate between fatigue as a mostly subjective trait value mainly occurring in clinical cohorts and objectively measurable state fatigue (hereafter referred to as *fatigability*) experienced by patients groups and by healthy subjects as well.

Due to the complex character of the fatigue construct and its versatile yet poorly understood neural causes, it is essential to diagnose and treat fatigue holistically.

Nevertheless, current clinical diagnostics almost solely rely on self-reported questionnaires (e.g., Modified Fatigue Impact Scale, MFIS or Fatigue Severity Scale, FSS) that exclusively assess the subjective experience of fatigue. To overcome this subjective diagnostic, recent research focuses on objective fatigability parameters. To investigate fatigability in healthy subjects and clinical cohorts, it is common to evaluate performance changes during sustained mental effort or after a prolonged time. Most of the time, those performance changes are quantified by an increase in reaction time and a decline in accuracy with time-on-task (Boksem et al., 2005; Hanken, Bosse, et al., 2016; Langner et al., 2010). However, especially in healthy cohorts, it is repeatedly shown that participants, besides their increasing feeling of subjective exhaustion, are often still able to maintain their behavioral performance (Crivelli et al., 2012; Le Mansec et al., 2019; Wascher et al., 2014). Furthermore, due to the repetitive nature of sustained mental tasks, participants' behavioral performance profits from learning effects, even though alternate versions of a task might reduce this effect. Therefore, other studies focused on electrophysiological indices and investigated brain wave activity alterations during an exhaustive task. The most prominent and reliable associations with fatigability are an increase in prefrontal theta (4-8 Hz, Barwick et al., 2012; Boksem et al., 2005; Craig et al., 2012; Lal & Craig, 2002; Wascher et al., 2014) and occipital alpha activity (8-12 Hz; Boksem et al., 2005; Craig et al., 2012; Wascher et al., 2014). Clayton et al. (2015) introduced an oscillatory model of sustained attention, in which frontomedial theta power supports cognitive monitoring and control processes while alpha power suppresses task-irrelevant processes. They postulate that when a person fatigues, the increase in frontomedial theta power may reflect the reactive engagement of theta-driven cognitive control processes via low-frequency phase synchronization. In contrast, the increase in alpha power over task-relevant cortical areas (e.g., occipital in a visual attention task) suppresses information processing and causes attentional deficits. Nevertheless, their model is derived from correlational studies, leaving causal associations between fatigability and task-dependent oscillatory alterations unknown.

However, fatigability-related oscillatory changes are assessed during the execution of the fatiguing task and are therefore directly related to the task type. Thus, they reflect a momentary state of exhaustion. And even though it is essential to examine fatigability and its effects on behavioral and electrophysiological parameters, it is also of

high primarily clinical relevance to find parameters that measure an underlying fatigue level and give an objective statement about how chronically exhausted a person is.

One possible approach is investigating cognitive top-down control processes that might be affected by chronically fatigue-induced cognitive deficits, such as extended tiredness, reduced working memory, concentration loss, and increased mind wandering (Boksem & Tops, 2008; Fisk et al., 1994). Regarding the pathogenesis of fatigue, many neuroimaging studies in clinical cohorts propose a malfunctioning cortico-striato-thalamo-cortical network centered on the thalamus (Chalah et al., 2015; Chaudhuri & Behan, 2000). Importantly, increased thalamus activity after inducing mental fatigability could also be found in healthy participants (Batouli et al., 2020). Since the thalamus is a pivotal hub of somatic and cortical afferences and efferences, this chronic malfunction in clinical cohorts or temporarily raised demand following mental fatigability in healthy participants may lead to permanent or temporary dysfunction of other cognitive control mechanisms processed by the thalamus. One of those mechanisms playing a pivotal role in cognitive control is sensory gating (Bak et al., 2014; Ji et al., 2013; Mayer et al., 2009). Sensory gating is an involuntarily and preconscious mechanism that protects the stimulus processing from disturbances and prevents the brain from an overload of irrelevant information. It can be assessed either by measuring the percentage of prepulse inhibition (PPI) or by measuring the suppression of the P50 event-related potential (ERP). PPI refers to a reduced muscular startle reflex to an intense (mostly acoustic) stimulus if a stimulus of lower intensity (prepulse) was previously presented. It is mostly referred to as sensorimotor gating. The P50 ERP, on the other hand, is evoked using the auditory paired click paradigm, where one click tone is followed by a second click tone approximately 500 msec after the first. In both paradigms, the first stimulus's processing suppresses the processing of the second, leading to a reduced reflex or amplitude to the second stimulus.

Sensory gating has already been shown to be impaired in various attention-related diseases such as schizophrenia (Patterson et al., 2008) and attention-deficit hyperactivity disorder (Holstein et al., 2013; Micoulaud-Franchi et al., 2015). One study by van der Linden et al. (2006) examined the effect of cognitive fatigability on sensorimotor gating. Therefore, PPI was assessed in healthy participants before and after a 90-minute continuous performance task (fatigue group) or a period in the waiting room (non-fatigue group). The authors found that fatigability significantly decreased PPI in the

fatigue group. Additionally, after the manipulation, sensorimotor gating ratios correlated negatively with the subjective feeling of exhaustion assessed by a visual analog scale. Another study by Aleksandrov et al. (2016) examined P50 suppression before and after the induction of physical fatigability in healthy participants. After physical exhaustion, participants had a significantly reduced or completely suppressed sensory gating index. However, to our knowledge, there are no studies on the effect of cognitive fatigue or fatigability on P50 sensory gating.

Therefore, in the present study, we assessed these gating parameters in healthy participants and evaluated sensory and sensorimotor gating changes after a fatigability-inducing task. Both parameters are computed before and after a 90-minute exhaustive task. Therefore, they are unrelated to the type of the fatiguing task.

As fatigue causes many car and work-related accidents (Caldwell et al., 2019; Philip, 2005) and severely reduces the quality of life for patients with fatigue (Fisk et al., 1994), it is of high relevance to find an efficient therapeutic treatment. One promising method that has mainly emerged in recent years is transcranial direct current stimulation (tDCS). TDCS is a form of non-invasive brain stimulation and delivers a mild constant current to the brain, thereby modifying neuronal membrane potentials. Via depolarization of resting membrane potentials (anodal tDCS), the electricity causes an enhancement of cortical excitability, while hyperpolarization (cathodal tDCS) leads to a decrement of cortical reactivity (Nitsche & Paulus, 2000). Interestingly, excitability-enhancing effects of anodal tDCS have been successfully demonstrated to outlast the stimulation period by several minutes to hours, proposed to result from long-term synaptic changes in the stimulated region (Liebetanz et al., 2002; Monte-Silva et al., 2013).

The majority of stimulation studies to counteract fatigability development applied anodal tDCS over the dorsolateral prefrontal cortex, as this area is shown to be most affected by fatigue (Borragán et al., 2018; Fiene et al., 2018; McIntire et al., 2014; McIntire et al., 2017; Nelson et al., 2014). In their study, Nelson et al. (2014) examined the influence of tDCS on vigilance decrement with time-on-task. Therefore, healthy participants were stimulated (1 mA for 10 min) over the left dorsolateral prefrontal cortex (DLPFC) either at the beginning or at the end of a 40-minute vigilance task. While participants who received sham tDCS experienced expected behavioral performance

decrements, anodal tDCS significantly affected reaction time, error rates, and blood hemodynamics. Likewise, McIntire et al. (2014; 2017) showed that anodal tDCS (2 mA for 30 min) over the left DLPFC was more beneficial than caffeine consumption in counteracting subjective state-fatigue and fatigability-related vigilance decrements during prolonged wakefulness of healthy participants. Furthermore, one clinical study by Fiene et al. (2018) aimed to investigate the effects of tDCS on fatigability development in patients with MS and showed a positive impact of anodal tDCS over the left DLPFC. They reported an increase in P300 amplitude and a reduced fatigability-related increase in reaction time with time-on-task compared to sham. In contrast to the previous studies, Borragán et al. (2018) showed no impact of anodal tDCS (1.5 mA for 25 min) in counteracting the behavioral effects of cognitive fatigability.

However, due to the aforementioned difficulties to validly operationalize fatigability utilizing behavioral changes and an increase in subjective exhaustion, a reliable validation of positive tDCS effects on fatigability remains difficult. Electrophysiological parameters unaffected by learning effects or other psychological biases (e.g., recall bias or social desirability) might be better markers for an objective validation of tDCS effects on fatigability.

In summary, the present study aimed to investigate i) the effects of fatigability on electrophysiological parameters and ii) their potential attenuation by prefrontal tDCS. Fatigability was induced by a 90-minute continuous performance task and conceptualized as a change in subjective fatigability ratings. We assigned all participants to either a verum or a sham control group. While the control group received sham stimulation, the verum group received 30 min anodal tDCS during the exhaustive task to counteract fatigability development. We hypothesized that 90 min of a continuous performance task lead to cognitive fatigability shown by an increase in subjective exhaustion as well as frontomedial theta and occipital alpha power. Additionally, increased fatigability will lead to a reduction of sensory and sensorimotor gating indices. Finally, we assumed that anodal tDCS positively affects fatigability-related changes and that this effect persists throughout the experiment and leads to smaller alterations in spectral measures and gating indices.

## 3.3.3 Materials and Methods

### 3.3.3.1 Participants

We initially recruited 60 participants. Due to technical problems during the experiment ($n$ = 3) or not completing the experiment ($n$ = 3), data of 54 participants have been assessed. Furthermore, 14 participants did not fulfill inclusion criteria for the gating analyses at baseline (see below). Thus, we enrolled 40 healthy participants (male = 10) in the age of 19 to 35 years ($M$ = 24.35 years, $SD$ = 3.97 years) in the final analyses. All participants were German native speakers and were pseudorandomly allocated to anodal ($n$ = 20) or sham tDCS group ($n$ = 20). Baseline group characteristics are listed in Table 5. Inclusion criteria were no history of neurological or psychiatric disorders, no tinnitus, no current depression (Beck-Depression-Inventory II, BDI-II < 13; Beck et al., 1996) or sleep disorder (Epworth-Sleepiness-Scale, ESS < 10; Johns, 1991), no auditory problems, and normal or corrected-to-normal vision. Additionally, participants had to meet tDCS criteria such as no cardiac arrhythmias or pacemaker, no pregnancy, no metal in the cranium except in the mouth (retainer), no surgical clips in or near the brain, no epilepsy or any kind of epileptic seizures in the lifetime, no recurring unexplained blackouts, and no chronic skin diseases on the should, face and scalp.

**Table 5.** *Baseline (BL) group characteristics.*

| | **anodal group ($n$ = 20) mean (± $SD$)** | **sham group ($n$ = 20) mean (± $SD$)** |
|---|---|---|
| gender f/m | 16/4 | 14/6 |
| age [years] | 23.40 (3.83) | 25.30 (3.98) |
| BDI [points] | 3.95 (2.84) | 4.90 (3.54) |
| ESS [points] | 5.65 (1.95) | 5.75 (2.10) |
| $PPI_{BL}$ [%] | 75.69 (18.13) | 79.47 (21.96) |
| $P50_{BL}$ [%] | 51.41 (30.22)* | 74.38 (21.07)* |
| $VAS_{BL}$ [points] | 35.86 (16.82) | 36.45 (18.53) |
| $IAF_{BL}$ [$\mu V^2$ x Hz] | 12.62 (9.94) | 10.00 (8.87) |
| $theta_{BL}$ [$\mu V^2$ x Hz] | 6.10 (5.05) | 5.26 (2.62) |

*BDI,* Becks Depression Inventory; *ESS,* Epworth Sleepiness Scale; *IAF,* individual alpha frequency; *PPI,* prepulse inhibition; *VAS,* visual analog scale; * $p$ > .05 two-sample t-test.

To ensure that participants were unaware of the study goals, participants were not instructed to restrict psychostimulants consumption on the day of the experiment, but consumption was assessed via questionnaire. Six participants in the anodal and nine participants in the sham group drank caffeine before the experiment, and two were smokers (one in each group). All participants were recruited from the University of Magdeburg in Germany and received monetary reward (Euro 30 in total) or course credit for participation in the study. The study was approved by the local ethics committee and was conducted in accordance with the Declaration of Helsinki.

#### 3.3.3.2 Gating paradigms

PPI was measured according to the guidelines of Blumenthal et al. (2005). Broadband white noise of 70 dB preceded the presentation of active stimuli by one minute and persisted as background noise during the entire PPI testing. The testing consisted of five 105 dB white noise bursts in the beginning that acted as habituation stimuli, followed by 60 randomly presented trials, each belonging to one of three possible conditions: (i) the prepulse-alone condition (80 dB 20 msec white noise bursts, 20 trials), which served as a baseline condition, (ii) the startle-alone condition (105 dB 40 msec white noise bursts, 20 trials) and (iii) the prepulse-startle condition (20 trials) in that the startle stimuli were presented 120 msec after the presentation of the prepulse stimuli. Both stimulus rise times were near-instantaneous. The inter-trial interval averaged 10 sec with a range from 8 to 12 sec.

To measure the P50 ERP, we used the standard paired-click paradigm (Light et al., 2010). The task consisted of 60 pairs of 80 dB white-noise clicks with a duration of 1 msec. The click pairs were presented with a 500 msec inter-click interval and a random 8 to 11 sec inter-trial interval. Before the stimulus presentation, the task began with 30 dB broadband white noise for one minute that preceded as background noise during the entire testing. Both testing sessions lasted approximately 10 min, during which the participants were to sit in an upright but relaxed position and focus on the white fixation cross located on the black background of a computer screen approximately 60 cm in front of them.

#### 3.3.3.3 Fatigability-inducing task

Fatigability was induced by letting participants complete an AX-continuous performance task (AX-CPT) for 90 min since this task has previously been successfully used to induce fatigability (Marcora et al., 2009; Pageaux et al., 2013; van der Linden et al., 2006). During this task, letters were sequentially presented one at a time on a black background in the center of a computer screen. One sequence of letters consisted of a red cue letter, two white distractor letters, and a red probe letter, all presented for a duration of 300 msec followed by 1200 msec inter-stimulus interval (cf. Figure 8B). Participants were asked to press, as quickly as possible, either the right or left CTRL-button. To a probe letter 'X' that followed a cue letter 'A' (AX-sequence), they were instructed to press the right CTRL-button. In any other possible sequences ['A' is followed by any other letter than 'X' (AY-sequence), 'X' followed any other letter than 'A' (BX-sequence), or both cue and probe letter were any letter but 'A' or 'X' (BY-sequence)] they were asked to press the left CTRL-button. Letter sequences were presented in a pseudorandom order, such that target sequences (AX) were presented at a 70 % frequency, while non-target sequences (AY, BX, BY) occurred at a 30 % frequency. Auditory feedback was provided for wrong and missed answers with a 500 Hz low-pitch tone through MS-TECH (LD-160) loudspeaker.

#### 3.3.3.4 Procedure

The study design is illustrated in Figure 8A. At the beginning of the testing, participants signed informed consent and completed several questionnaires to assess their ability to participate in the experiment (tDCS questionnaire), their current mood (BDI-II), and their daytime sleepiness (ESS). After electroencephalogram (EEG) mounting, the experiment started with either the PPI or P50 paradigm, in a randomly chosen order. After the first presentation of the PPI- and P50-task ($PPI_{BL}$, $P50_{BL}$), the participant received instruction for the following 90-min AX-CPT task and performed one training block consisting of 10 trials. The main AX-CPT task consisted of six blocks (B1-B6) of 15 min each. Before the first (VAS at baseline) and after each block, participants were asked about their current subjectively perceived fatigability status. Therefore, participants reported how mentally exhausted they felt "right now at this moment" on an electrical visual analog scale (VAS) from 0 to 100. A 90 sec break followed the answer.

The tDCS/sham stimulation was applied in the third and fourth block of the AX-task. To ensure blinding and the same procedure in both groups, we applied sham stimulation in the first-to-second block and the fifth-to-six block (cf. Figure 8A). During B1, B2, B5, and B6, all participants received sham stimulation. Participants in the verum group received 30 min of anodal tDCS during B3 and B4, while participants in the placebo group received 30 min of sham tDCS during B3 and B4. To restart the stimulation after every two blocks, the experimenter had to quickly enter the testing room but ensured not to engage in any conversation (see below for further information). Directly after the AX-CPT task, PPI and P50 were assessed for the second time ($PPI_{post}$, $P50_{post}$) in the same order as they were presented at baseline. Subsequently, participants completed a short questionnaire for tDCS side effects (see below) and were then debriefed about the experiment.

A single test session lasted about four hours. To counteract changes in subjective fatigability and cognitive performance caused by the time of day (Claros-Salinas et al., 2010; Kumari et al., 2009; Morris et al., 2002), all testing sessions started before noon.

#### 3.3.3.5 Stimulation

For tDCS application, the anode (5x7 cm) was placed over the left DLPFC corresponding to the F3 electrode of the international 10-20 EEG system (Jasper, 1958). The reference electrode (5x10 cm) was placed extracephalically over the right shoulder (cf. Figure 8C). This arrangement was chosen because it could already successfully counteract fatigue-related attention deficits (Fiene et al., 2018; Mattioli et al., 2016). Additionally, according to the specifications of Nasseri et al. (2015), a unilateral monopolar electrode placement prevents unwanted cephalic polarization effects under the return electrode. A battery-driven DC stimulator (DC Stimulator Plus, NeuroConn, Germany) delivered the stimulation using two rubber electrodes covered with saline-soaked sponges. Direct current was applied with an intensity of 1.5 mA with a 15 sec fade in/out time. The impedance of stimulation electrodes was kept below 10 kΩ. For anodal stimulation, the direct current was applied for 30 min. For sham stimulation, the 15 sec fade-in – 30 sec short stimulation – 15 sec fade-out approach (Ambrus et al., 2012) was used. This procedure ensured that all participants experienced the initial itching sensation that

recedes over the first seconds of tDCS and guaranteed successful blinding (Ambrus et al., 2012; Gandiga et al., 2006).

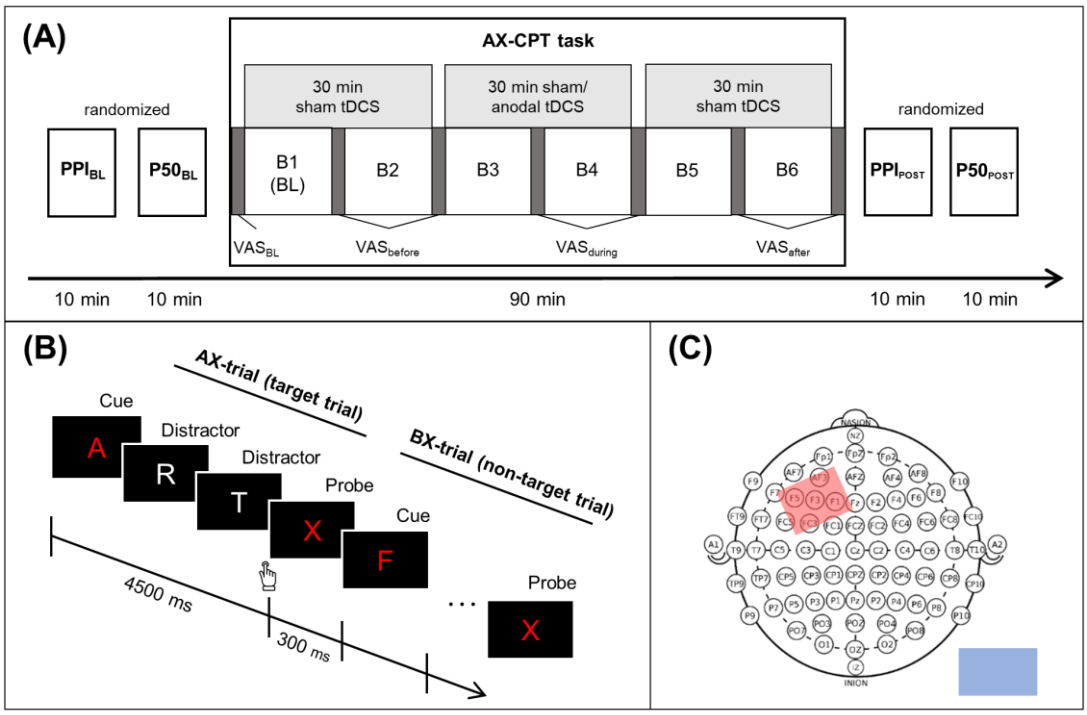


**Figure 8.** Experimental procedure and electrode setup. (A) Study design: prepulse inhibition (PPI) and the event-related potential P50 were assessed as baseline (BL) measures, using two auditory paradigms in a randomly chosen order ($PPI_{BL}$, $P50_{BL}$). After a 90-min continuous performance task (AX-CPT task) that consisted of six blocks (B1-B6) of 15 min each, both paradigms were assessed again ($PPI_{post}$, $P50_{post}$). B1 of the AX-CPT task was used as a baseline measure. Before the first and after each block, participants were asked about their current subjectively perceived fatigability status on a visual analog scale ($VAS_{BL}$ - $VAS_{B6}$). VAS scores after B1 and B2 (*before*), B3 and B4 (*during*), and B5 and B6 (*after* stimulation) were averaged, respectively. During B1, B2, B5, and B6, all participants received sham stimulation. In B3 and B4, participants in the anodal group received 30 min of anodal transcranial direct current stimulation (tDCS), while participants in the placebo group received 30 min of sham tDCS. (B) AX-CPT task: single letters are visually presented as a series of cue-distractor-distractor-probe pairs. A target is defined as the occurrence of an 'X' probe following an 'A' cue. Non-target trials are either BX, AY, or BY trials (where B refers to any non-A cue, and Y refers to any non-X probe). At the display of the probe letter, the participant is asked to press either the right CTRL-button in case it is a target trial or the left CTRL-button in a non-target trial. (C) Stimulation design: the active electrode (5x7 cm) was placed over the left dorsolateral prefrontal cortex, and the reference electrode (5x10 cm) was placed over the right shoulder.

To test for tDCS side effects and ensure blinding, participants were asked to fill out a short questionnaire at the end of the experiment. The questionnaire was designed according to the consensus tDCS guidelines (Antal et al., 2017) and asked whether and to what extent participants felt any of the following sensations due to stimulation: headache, nausea, dizziness, loss of concentration, fatigue, metallic taste, skin irritation or itch, prickle or heat on the scalp. The numeric rating scale ranged from 0 = no sensation to 3 = strong sensation. Analysis of this questionnaire using Mann-Whitney U tests did not reveal differences between both groups (all *ps* > .178), indicating successful blinding.

#### 3.3.3.6 EEG signal recording and preprocessing

EEG was recorded at F1, F2, Fz, Cz, Pz, POz, P3, PO3, P4, PO4 (corresponding to the international 10-20 system; Jasper, 1958) using Ag/AgCl-electrodes mounted in an elastic cap (EasyCap GmbH, Germany). The ground electrode was attached to the AFz position, and all channels were referenced to the left and right mastoid. The electrooculogram (EOG) was recorded using two electrodes placed below the pupil (vertical EOG) and to the external canthus of the left eye (horizontal EOG). To measure the startle response with electromyography recording (EMG), two electrodes were placed over the right orbicularis oculi muscle. The ground electrode was placed on the forehead. The data was recorded by Brain DC amplifier (Brain Products, Germany) and the corresponding software (BrainVision Recorder, version 1.20, Brain Products, Germany) sampled at 1000 Hz. The online band-pass was 0.01 to 250 Hz with a notch filter at 50 Hz. Impedances were kept below 5 kΩ. EEG preprocessing and data analysis were carried out in BrainVision Analyzer 2.1 (Brain Products, Germany).

For the P50 analysis, the EEG data were epoched from -150 to 499 msec post stimulus and then offline band-pass filtered from 1 to 47 Hz. The data was then baseline corrected (-50 to 0 msec) and corrected for eye-movement artifacts using the Gratton and Coles method (Gratton et al., 1983). Before averaging, epochs were manually inspected and rejected if they contained substantial artifacts. The epoched data were then averaged for the first and the second stimulus separately. The peak detection of the auditory evoked P50 potential was measured at channel Cz. Peaks were chosen based on the following criteria from Mann et al. (2008): (i) the P50 peak was first identified as the most positive peak occurring 30 to 80 msec after the stimulus, (ii) the P50 peak needed to be preceded

by a negative (Na) and a positive deflection (Pa), (iii) and the P50 peak to the second stimulus had to occur within ± 10 msec around the latency of the prior detected P50 peak of the first stimulus. The P50 amplitude was defined as the difference between the P50 peak and the preceding negative trough, separately for the first and second stimuli. If there was no P50 peak in that range, the P50 amplitude of the second stimuli was scored as 0.01. Subsequently, the P50 suppression percentage was calculated according to the following formula:

$$(1 – S2 / S1) * 100$$

S1 is the P50 amplitude to the first and S2 the amplitude to the second stimulus. Accordingly, higher P50 suppression ratios indicate higher sensory gating. If P50 suppression was negative (the amplitude to the second stimulus greater than the amplitude to the first stimulus), it was scored as zero. Four participants had to be excluded from further data analyses for not showing an identifiable P50 waveform at baseline.

For the PPI analysis, the EMG data were band-pass filtered from 28 to 400 Hz with an additional notch filter of 50 Hz. For each participant, startle responses were segmented for each trial type (-100 to 200 msec after stimulus onset) and then baseline corrected (-100 to 0 msec). Subsequently, the EMG signal was rectified and smoothed with a moving average at a time constant of 11. A manual visual inspection followed, in which all trials featuring excessive noise or a spontaneous blink in the period immediately preceding the stimulus onset were excluded from further analysis. For each trial, the startle response was considered as the maximum blink amplitude in a response window from 20 to 120 msec after stimulus onset. As van der Linden et al. (2006), we defined a valid startle response as a peak of at least 3 SD above baseline activity, with baseline activity calculated as the average response to the prepulse in the prepulse-alone trials, except for those trials in which the startle activity caused by the prepulse exceeded 10 μV. Participants who reacted in half or more trials to the prepulse in the baseline measurement were excluded entirely. This lead to the exclusion of three participants from the further data analyses. Additionally, seven participants were classified as non-responders and therefore excluded because they exhibited startle responses in less than half of the startle-alone trials during baseline (Blumenthal et al., 2005). The percentage of PPI was calculated according to the following formula:

$$((M_{startle\text{-}alone} - M_{prepulse\text{-}startle}) / M_{startle\text{-}alone}) * 100$$

Accordingly, higher PPI percentages indicate higher sensorimotor gating. If the PPI percentage was negative, hence, the mean response to the startle stimuli after the prepulse stimuli was greater than the mean response to the startle stimuli only, it was scored as zero. We report peak magnitude, meaning the average includes values of zero for nonresponses.

To spectrally analyze the EEG data during the AX-CPT task, the signal was band-pass filtered from 0.3 to 40 Hz, resampled to 256 Hz, and then corrected for eye-movement artifacts using the Gratton and Cole method (Gratton et al., 1983). Subsequently, 2 sec long segments with an overlap of 200 msec were extracted from the continuous EEG in the experimental blocks. To avoid tDCS-induced stimulation artifacts, we analyzed the total power of the last 10 min of the first block as a baseline and the second, fifth and sixth block (because no stimulation was applied during these time spots). The resulting segments were submitted to a fast Fourier transformation, using a Hanning window with 10 % of the total segment length. After averaging, we determined individual baseline alpha peak frequencies as peaks in the range of 8 to 12 Hz and defined the individual alpha and theta ranges according to Klimesch (1999). Hence, occipital alpha power was defined as the area band value sum between -1 Hz and +1 Hz of the individual alpha frequency, while frontomedial theta power was defined as the area band value sum in the range of -6 Hz to -4 Hz of the individual alpha frequency. Changes over time-on-task were then examined for the individual frequency bands.

#### 3.3.3.7 Statistical analysis

For each participant, we assessed subjective (VAS scores) and objective (PPI and P50 gating as well as alpha and theta power) fatigability values. We operationalized fatigability as relative performance decline over time-on-task (Kluger et al., 2013). Accordingly, we assessed fatigability parameters in relation to baseline performance. For the VAS analysis, we additionally averaged VAS scores that were assessed *before* (after B1 and B2), *during* (after B3 and B4), and *after* (B5 and B6) tDCS (cf. Figure 8A). For the analysis of the alpha and theta power, spectral data during the last 10 min of B2, B5, and B6 in relation to the last 10 min of B1 (baseline) were analyzed. To reduce the impact of extreme values on the analysis without losing data, we winsorized all outliers in the

fatigability indices by 90 %. Therefore, we reduced or raised outlying high or low values in magnitude to a value that is still at the high (95$^{th}$ percentile) or low end (5$^{th}$ percentile) of the distribution, but not as extreme (Field, 2013). A value was defined as an outlier if it differed by twice the standard deviation or more from the mean.

For the VAS scores and the spectral data, we performed repeated measures analysis of variance (ANOVAs) with Greenhouse-Geisser correction, if necessary. PPI and P50 data were analyzed using *t*-tests. Additionally, explorative correlation analyses were performed.

Baseline differences between both groups were evaluated with independent samples *t*-tests (cf. Table 5). Groups differed in P50 suppression only with higher suppression ratios in the anodal group [$t(38) = 2.789$, $p = .008$, $d = .882$].

## 3.3.4 Results

### 3.3.4.1 Fatigability manipulation

First, we verified that the fatigability manipulation was, in fact, subjectively exhausting. We entered VAS scores into a 3 x 2 ANOVA with the within-subject factor *time* (before, during, after) and the between-subject factor *stimulation* (anodal, sham). As expected, the continuous performance task significantly increased subjective exhaustion [main effect of the factor *time*: $F(1.700 , 64.600) = 55.030$, $p < .001$, $\eta_p^2 = .592$]. However, we did not find a main effect of the factor *stimulation* [$F(1,38) = .266$, $p = .609$], nor a significant interaction [$F(1.700 , 64.600) = 1.668$, $p = .200$]. As shown in Figure 9, in both groups participants' subjective exhaustion increased monotonically over the experimental blocks.

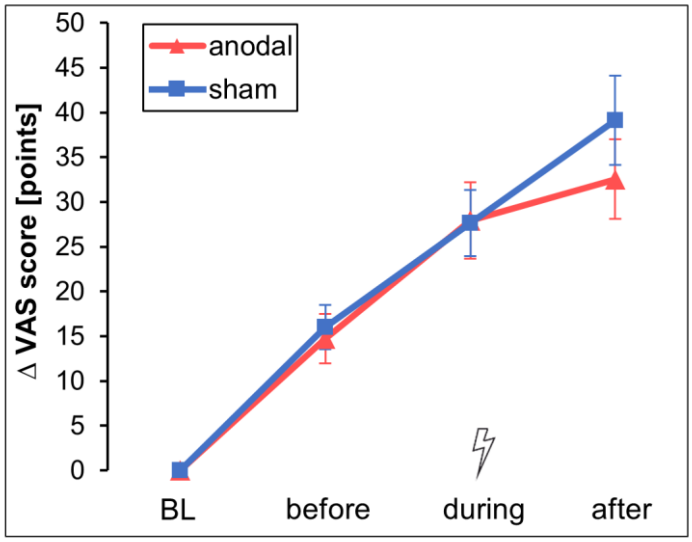


**Figure 9.** Modulation of subjective exhaustion scores for sham and anodal group relative to baseline (BL) as a function of time-on-task. BL reflects the baseline value assessed before the fatiguing task, before the mean change in fatigability scores after block 1 and 2 relative to baseline, during the mean change after block 3 and 4 (stimulation blocks, where the anodal group received 30 min of anodal transcranial direct current stimulation), and after reflects the mean change after block 5 and 6.

### 3.3.4.2 Spectral power changes during the fatiguing task

Difference scores for frontomedial theta and occipital alpha power were separately entered into 3 x 2 ANOVAs with the within-subject factor *time* (B2, B5, B6) and the between-subject factor *stimulation* (anodal, sham). Frontomedial theta power over the electrode Fz increased significantly with time-on-task [main effect of the factor *time*: $F(1.452 , 55.193) = 14.451$, $p < .001$, $\eta_p^2 = .276$]. Neither the main effect of the factor *stimulation* [$F(1,38) = .873$, $p = .356$] nor the interaction reached significance [$F(1.452 , 55.193) = 1.242$, $p = .287$], indicating that tDCS had no effect on theta power changes over time-on-task. As illustrated in Figure 10A and 10B, theta power increased in both groups from baseline to the end of the task.

Explorative correlation analyses showed that the total increase of theta power (B6) correlated with the total increase of subjective exhaustion (VAS after) over both groups ($r_s = .343$, $p = .030$, uncorr.). Thus, the increase of subjective exhaustion with

time-on-task was associated with increased frontomedial theta power with time-on-task. No further correlations reached significance (all *ps* > .223).

Individual occipital alpha power over electrode POz significantly increased with time-on-task [main effect of the factor *time:* $F(2,76) = 19.439$, $p < .001$, $\eta_p^2 = .338$]. Analysis revealed no significant main effect of the factor *stimulation* [$F(1,38) = .577$, $p = .452$], but a significant *time* x *stimulation* interaction [$F(2,76) = 5.308$, $p = .007$, $\eta_p^2 = .123$] (cf. Figure 10C and 10D).

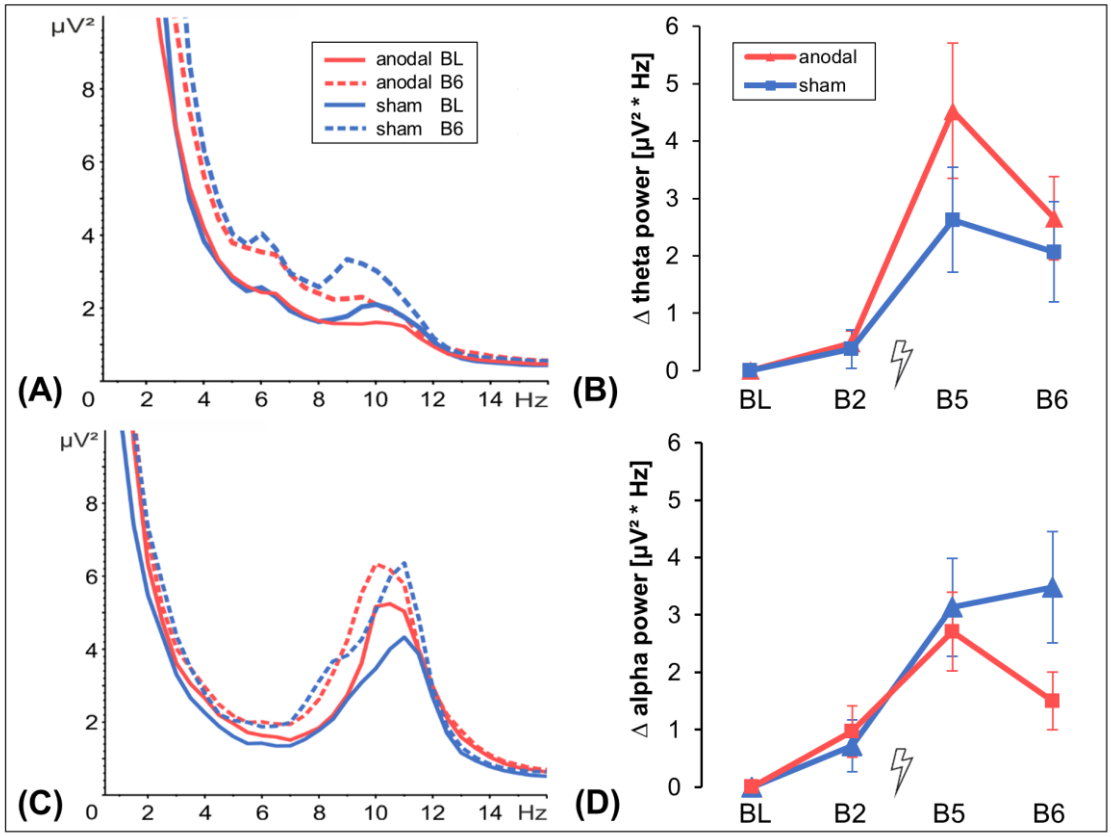


**Figure 10.** Power spectra for theta power over Fz (A) and alpha power over POz (C) during block 1 as baseline value (BL) and block 6 (B6) in the continuous performance task separate for sham and anodal group. The anodal group received 30 min of anodal transcranial direct stimulation during B3 and B4. Additionally, B+D depicts the average change in theta (B) and alpha activity (D) for sham and anodal group relative to baseline (BL).

Post-hoc tests using Bonferroni-correction revealed a significant alpha increase with time-on-task (B2 vs. B6) in the control group [$t(19) = 5.562$, $p < .001$, $d = .879$] but not in the anodal group [$t(19) = 1.069$, $p = 1.000$]. Thus, while there was a similar alpha increase during block B2 and B5, alpha power during B6 was significantly reduced in the anodal group compared to the placebo group [$t(38) = 1.693$, $p = .049$, uncorr.].

Further, explorative analysis of possible associations between the total increase of alpha power and the remaining fatigability parameters did not reveal any significant relationships neither over both groups (all *ps* > .230) nor in the separate groups (sham: all *ps* > .223; anodal: all *ps* > .082).

To summarize, our findings demonstrated that time-on-task had a significant effect on subjective exhaustion. Furthermore, we confirmed a fatigability-related increase in frontomedial theta and occipital alpha power. Anodal tDCS over the dorsolateral prefrontal cortex successfully counteracted the increase in occipital alpha power.

#### 3.3.4.3 Sensorimotor and sensory gating changes

Fatigability significantly decreased PPI ratios in the control group [$t(19) = -2.903$, $p = .009$, $d = -.649$] by 4.96 (± 7.64) %, while in the anodal group PPI remained stable (non-significantly decrease by 1.93 (± 7.43) %; [$t(19) = -1.160$, $p = .260$]) (cf. Figure 11C). To investigate whether the fatigability manipulation already affected basic startle amplitude, additional t-tests on startle amplitudes in startle-alone trials were performed. In the control group, startle amplitudes increased significantly from baseline to post [$t(19) = 2.378$, $p = .028$, $d = .532$], but not in the anodal group [$t(19) = .277$, $p = .785$]. When investigating the startle amplitude in prepulse-startle trials, the analysis revealed significantly increased startle amplitudes from baseline to post in both groups [sham: $t(19) = 4.110$, $p < .001$, $d = .919$; anodal: $t(19) = 3.118$, $p = .006$, $d = .697$]. Hence, the effect of fatigability on PPI in the control group is not due to habituation effects to the startle response, but rather driven by an increased startle amplitude in prepulse-startle trials (cf. Figure 11A & B). Correlational analysis for PPI changes with the remaining fatigability parameters did not reveal any significant associations (all *ps* > .094).

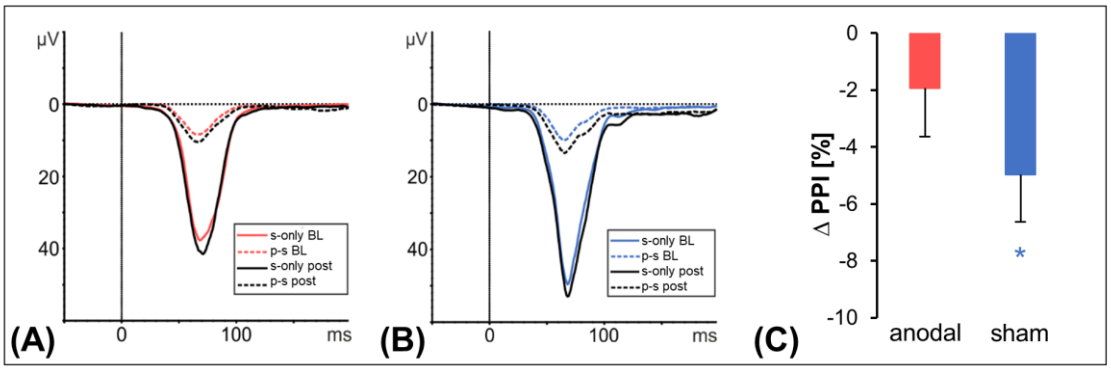


**Figure 11.** Mean magnitudes of the acoustic startle responses elicited by the startle-only (s-only) and prepulse-startle trials (p-s) for anodal (A) and sham (B) group at baseline (BL) and post-test. The anodal group received 30 min of anodal transcranial direct current stimulation during the exhaustive task that was carried out between PPI pre- and post-test. Figure 11c depicts the average change in PPI percentage (prepulse inhibition) for sham and anodal group relative to baseline. * $p < .05$.

Finally, we investigated changes in P50 sensory gating (cf. Figure 12). While in the control group, sensory gating decreased significantly by 14.56 % [$t(19) = -2.739$, $p = .013$, $d = -.613$], there was no significant change after anodal stimulation [non-significantly increase by 1.79 %; $t(19) = .232$, $p = .819$] (cf. Figure 12C). In addition, we further analyzed if the effect of fatigability on P50 gating in the control group was systematically driven by a decrease of the P50 ERP response to the first (S1) or second (S2) stimulus. However, both stimuli were not differently affected by fatigability (all $ps > .332$). Correlational analysis for changes in P50 gating with the remaining fatigability parameters did not reveal any significant associations (all $ps > .082$).

To summarize, fatigability systematically affected both gating parameters and significantly reduced the PPI and P50 sensory gating. Anodal tDCS over the left dorsolateral prefrontal cortex counteracted fatigability development and reduced the decline in gating parameters.

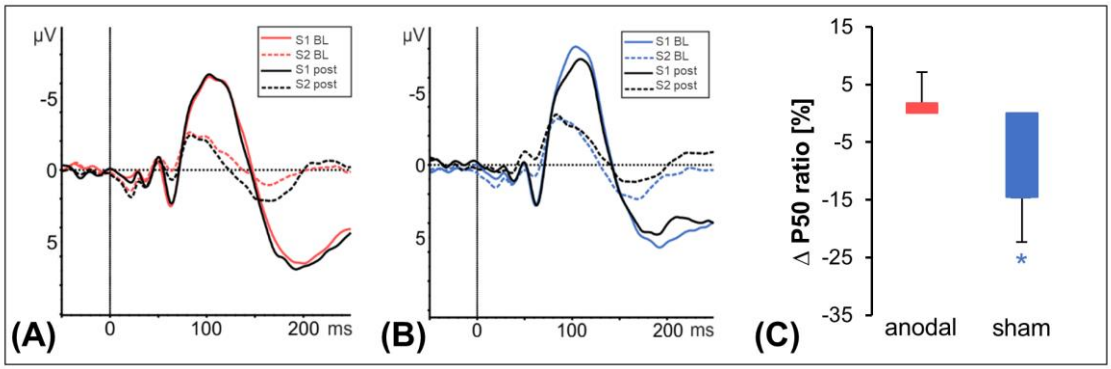


**Figure 12.** Grand mean ERP waveforms at Cz to the first (S1) and second (S2) click for anodal (A) and sham (B) group at baseline (BL) and post-test. The anodal group received 30 min of anodal transcranial direct current stimulation during the exhaustive task that was carried out between P50 pre- and post-test. Figure 12c depicts the average change in P50 gating ratios for sham and anodal group relative to baseline. * $p < .05$.

### 3.3.5 Discussion

The aim of the present study was i) to complement the purely subjective fatigue diagnostic with objective electrophysiological fatigability parameters, and ii) to prove the potential therapeutic application of tDCS for a fatigability intervention. Therefore, in healthy subjects, we induced fatigability with a 90-min continuous performance task. This manipulation reliably induced subjective exhaustion. Furthermore, we confirmed fatigability-related increases in frontomedial theta and occipital alpha power throughout the task. Additionally, fatigability systematically affected gating parameters, assessed independently of the exhaustive task. Fatigability significantly reduced PPI as well as sensory gating. Anodal tDCS over the left DLPFC successfully counteracted fatigability development and reduced the fatigability-related increase in occipital alpha power as well as the decline in gating parameters.

In line with our hypothesis, our data confirmed a fatigability-related increase in frontomedial theta and occipital alpha power in the control group. Importantly, we demonstrated that a single session of prefrontal tDCS attenuated the increase of occipital alpha power. We assume that this positive tDCS effect can be explained by the fact that anodal stimulation over the left DLPFC leads to an increase of prefrontal theta power, as has been previously shown in other studies (Mangia et al., 2014; J. Miller et al., 2015;

Zaehle et al., 2011). As previously described, Clayton et al. (2015) accentuated the role of frontomedial theta power in compensatory control mechanisms to enlarge top-down control processes in a fatiguing brain. They suggest that frontomedial theta power reflects the detection of a mismatch between current and desired levels of attention. The positive correlation between the increase of theta power and subjective feeling of fatigue in our study further supports this assumption. Theta-driven cognitive control processes communicate with posterior cortical areas via low-frequency phase synchronization and suppress inhibitory occipital alpha power (Clayton et al., 2015). This assumption is further supported by studies that find an anticorrelation between prefrontal theta and occipital alpha power (Mathewson et al., 2014; Mazaheri et al., 2009; Mazaheri et al., 2010). However, in the present study, we were unable to find direct tDCS effects on theta power. To avoid stimulation artifacts, we only collected theta power in those blocks, where no stimulation was applied. Hence, it might be possible that theta activity increased in the anodal group but that this increase quickly attenuated after the stimulation. J. Miller et al. (2015) investigated tDCS-induced changes in frontal-midline theta activity. Therefore, anodal stimulation was applied over the prefrontal cortex (1 mA for 15 minutes), and theta power was assessed in a resting-state period immediately after the stimulation as well as in a cognitive task that followed the resting state period. The authors found a significant increase in theta power directly after the stimulation, but this increase was quickly dissipated before participants completed the cognitive task. Therefore, we can only assume that the positive tDCS effect on the fatigability-related alpha increase was due to a tDCS-induced increase in frontomedial theta power.

For future studies, transcranial alternating current stimulation (tACS) might be a better alternative non-invasive brain stimulation method to improve theta-driven control processes selectively. With tACS, an alternating current is applied to the scalp, which modulates ongoing oscillations by causing neural activity entrainment to the externally applied current (see Reato et al. (2013) for a comprehensive review). Hence, it is possible to stimulate the brain in the targeted frequency selectively (e.g., theta-tACS) and investigate the causal role of theta-driven monitor functions. However, to our knowledge, theta-tACS was not applied to investigate the effects on fatigue or fatigability so far. Furthermore, it is of particular interest to examine how theta-driven control processes are altered in clinical patient groups with chronic fatigue. Due to the malfunctioning cortico-

striato-thalamo-cortical fatigue network, fatigability could primarily be driven by the lack of theta-driven control functions. Thus, patients with chronic fatigue may particularly benefit from external stimulation.

In summary, spectral measures, such as frontomedial theta increase and occipital alpha increase, have repeatedly been reported to be good indices for fatigability. In the present study, we confirmed the fatigability-related theta and alpha power increase and showed that tDCS can counteract this fatigability-related increase in occipital alpha power. We propose that especially occipital alpha power is a promising electrophysiological parameter to evaluate fatigability severity and additionally objectively validate tDCS effects.

#### 3.3.5.1 Gating deficits as fatigability markers

As predicted, we demonstrated decreased sensorimotor and sensory gating in the sham group. Additionally, this effect was not due to habituation to the startle stimuli, in that the startle response rather increased at post- compared to the pre-test. Moreover, the effect was explicitly driven by an increased response to the startle stimuli after a prepulse was presented. Likewise, there was no habituation effect to the first click stimuli in the P50 paradigm.

Our data are generally in line with the study from van der Linden et al. (2006), who also reported significantly decreased PPI after fatigability induction. However, while we found a mean decrease of 5 % PPI in the control group, they found a much greater decrease of approximately 20 %. This might be the result of gender differences in PPI. Thus PPI has repeatedly been more prominent in male participants (Swerdlow et al., 1993; Swerdlow et al., 2016) and might also be more affected by fatigability. While van der Linden et al. (2006) only examined male participants, we also included females in our sample, which might have resulted in the attenuated PPI decreases. However, with fatigue having such a significant impact in clinical populations, which in the case of patients with MS, two-thirds of the population consists of females, and since one of the main goals of this study was to investigate reliable fatigability parameters that can later be applied in such clinical samples, we decided to include both genders for a representative sample.

To the best of our knowledge, this is the first study providing evidence for the effects of cognitive fatigability on P50 sensory gating. Thus, while Aleksandrov et al. (2016) reported P50 gating deficits after physical exertion using a muscle load task with sustained contraction as the fatigability-inducing task, our data show that P50 gating is also suppressed after a cognitively exhausting task. Keeping in mind that many car- and work-related accidents are primarily due to cognitive fatigue and fatigability, this is an important finding that should be considered in future prevention studies.

Additionally, we showed that when anodal tDCS was applied during the fatiguing task, deficits in both gating parameters attenuated. This is consistent with the existing literature that reports a malfunctioning cortico-striato-thalamo-cortical fatigue network in clinical cohorts chronically suffering from fatigue (Chalah et al., 2015; Chaudhuri & Behan, 2000) but also studies that found altered thalamus activity after inducing mental fatigability in healthy participants (Batouli et al., 2020). Hence, this change in thalamus activity may lead to permanent or temporary dysfunction of thalamus-processed cognitive control mechanisms such as sensorimotor and sensory gating. Even with the exact effect mechanisms not fully understood, tDCS positively affects the communication between all areas belonging to the fatigue network and thereby improves fatigue-related decrements. Our results confirm this positive impact of prefrontal tDCS on the entire fatigue network and show that one single session of prefrontal tDCS could improve fatigability-related gating deficits.

In sum, our results support PPI and P50 sensory gating deficits as objective and task-independent fatigability parameters. They have already been repeatedly reported to be valid and reliable diagnostic parameters for other attention-related deficits (Holstein et al., 2013; Micoulaud-Franchi et al., 2015; Patterson et al., 2008). Additionally, objective electrophysiological parameters that are independent of learning effects or psychological biases are of high predominantly clinical relevance to give an objective statement about how severe a patient is suffering from chronic fatigue. Both gating paradigms for detecting PPI and P50 are safe and methodically simple neuropsychological methods that could easily be integrated into the fatigue diagnostic.

#### 3.3.5.2 Missing tDCS effects on subjective exhaustion

Contrary to our previous assumption, we could not find a positive tDCS effect on subjective exhaustion. Thus, in both groups, subjective fatigability increased with time-on-task.

Our initial hypothesis was based on clinical studies that found fatigue improvement after tDCS over DLPFC in patients with MS (Ayache et al., 2017; Chalah et al., 2020; Chalah, Riachi, et al., 2017; Charvet et al., 2018). However, there are several difficulties with this assumption. First, in patients with MS, the pathophysiology underlying chronic fatigue/fatigability might substantially differ from that of healthy participants who experience fatigability due to an exhaustive task. Hence, Saiote et al. (2014) investigated tDCS effects on subjective fatigue in patients with MS and showed that the individual lesion load within this cortical area scaled the effectiveness of left prefrontal tDCS. Patients with a higher lesion load responded more positively to anodal tDCS. In healthy participants, however, fatigability does not result from a lesion-related underactivity of the prefrontal cortex but rather from a momentary malfunctioning top-down control of the frontal cortex and/or inhibitory alpha oscillations. Therefore, even if the exact underlying pathology remains unclear, study results from clinical cohorts may not be directly applicable to fatigability in healthy individuals. Second, all of the aforementioned studies investigated trait-fatigue rather than state-fatigue and used repetitive stimulations. Hence, chronic fatigue over a more extended period (usually 1-2 weeks) rather than 90 minutes was sampled, and participants were usually stimulated five times on five consecutive days. This again highlights the importance of a unified fatigue taxonomy. Thus, it makes a tremendous difference whether one examines chronic fatigue in clinical cohorts as a trait-component or state-fatigue (fatigability) that can be found in clinical and healthy cohorts (Linnhoff et al., 2019).

Fiene et al. (2018) investigated tDCS effects on state-fatigue in patients with MS and retrieved the subjective exhaustion at several time points throughout the test session. However, while they were able to find positive tDCS effects on P300, they did not find subjective fatigability improvements. On the contrary, McIntire et al. (2014; 2017) found positive tDCS effects on subjective fatigability development in healthy subjects. However, their study design differs from other fatigue studies in that fatigability

was induced by a period of extended wakefulness as opposed to an exhaustive task, as it is common in other fatigability studies. Additionally, they stimulated with 2 mA rather than 1.5 mA as in our study or the study from Fiene et al. (2018), which might have enhanced tDCS effects. Charvet et al. (2018) reported besides positive tDCS effects on trait-fatigue additionally that tDCS was able to improve state fatigue ratings after each tDCS session. However, they only reported the average pre- and post-ratings with all study sessions combined. It remains unclear if one study session was already able to create this improvement or if repetitive stimulation sessions were necessary. Thus, while a single session of anodal tDCS can improve objective fatigability parameters, it might need multiple repetitive tDCS sessions to induce cumulative changes in the fatigue network, thereby evoking a subjectively perceivable change in the feeling of fatigability.

Moreover, it could be possible that our unilateral monopolar electrode placement was inefficacious regarding a subjective fatigue improvement. We opted for this design because the extracephalic reference electrode placement prevents unwanted cephalic polarization effects under the return electrode and, in addition, avoids interference of the tDCS electrodes with the EEG measurement. However, while as in our study, Borragán et al. (2018) and Fiene et al. (2018) used an extracephalic reference electrode and did not find subjective fatigue improvements, some studies demonstrated a positive effect of tDCS on the subjective fatigue in patients with MS using a bilateral electrode montage (Ayache et al., 2017; Chalah et al., 2020; Chalah, Riachi, et al., 2017; Charvet et al., 2018). However, at least two studies (McIntire et al., 2014; McIntire et al., 2017) also demonstrated positive tDCS effects on subjective fatigue with an extracephalic reference montage. Thus, while some studies might indicate a systematic effect of the electrode montage, also contrary data exist.

Furthermore, it might be possible that the repetitive character of the VAS recordings throughout the test session made our participants aware of the task's meaning and lead to socially desirable answers. Thus, participants either intentionally answered that they were less exhausted than they were (in favor of the experimenter) or were guided by their previous answers and automatically responded a little worse every time. In retrospect, we might instead recommend reducing the retrieval of VAS scores to a minimum of before and after the exhaustive task or mask the purpose with additional VAS scales.

Interestingly, explorative analyses showed a correlation between the overall increase in theta power and the overall increase in subjective exhaustion. At the same time and in contrast to the remaining objective fatigability parameters, only theta power and subjective exhaustion were unaffected by the anodal tDCS. The exact nature of the relationship between objective fatigability and subjective exhaustion remains a general and still open question. One reason for the frequently observed divergence between subjective and objective fatigue parameters might be related to the parameters' pure subjective nature detectable via introspection only. Furthermore, considerable high heterogeneity in the taxonomies and scales used for assessing subjective cognitive fatigue exists (see Linnhoff et al. 2019 for a recent overview). Thus, in fatigue research, various self-created VAS scales are applied that could be interpreted differently by the participants. It remains difficult to conclude whether the evaluated electrophysiological changes can be associated with the subjective feeling of exhaustion or whether they are individually observable phenomena with different causes.

In summary, the difficulties mentioned above demonstrate the importance of objective parameters for the valid recording of tDCS effects on fatigability. However, without improving subjective exhaustion, one cannot say that tDCS has a positive effect on fatigue and fatigability. Both constructs are difficult to differentiate from other attention-related processes if not subjectively felt. It is questionable to what extent one can objectively improve fatigue or fatigability if the subjects are not subjectively less exhausted. Therefore, further studies should consider repetitive stimulations to improve fatigue or fatigability objectively and positively impact the subjective feeling. However, this creates methodical difficulties, such as that those long repetitive testing sessions reduce compliance, increase psychological biases, and ensure learning effects.

#### 3.3.5.3 Limitations

In our study, we decided not to instruct participants to restrict psychostimulants consumption on the day of the experiment. Since we were interested in the subjective perception of fatigue/fatigability and its changes with time-on-task as well as its relation to objective fatigability parameters, it was essential for us to keep participants ignorant of the study goals. Furthermore, it has been shown that nicotine deprivation may reduce cognitive functions and task-related neurophysiology (Grundey et al., 2015; Grundey et

al., 2017). However, psychostimulants could have influenced fatigability. Nevertheless, additional analyses revealed no baseline differences between participants that consumed caffeine before the experiment and those who did not.

Additionally, since our control analysis showed a significant group difference in the P50 baseline data, we cannot exclude that baseline variations might have influenced an effect following the intervention. Accordingly, we refrained from directly comparing the P50 values between the stimulation groups but focused on direct changes within the group parameters.

#### 3.3.5.4 Conclusion

Our results show that task-induced fatigability leads to systematic changes in objective electrophysiological parameters (frontomedial theta and occipital alpha power as well as gating parameters). Furthermore, a single session of anodal tDCS over the left dorsolateral prefrontal cortex prevented fatigability-related increases in occipital alpha power and gating deficits. Our data suggest that occipital alpha power, as well as sensorimotor and sensory gating, can represent an extension of the currently very subjective fatigue diagnostic in a clinical setting. These objective electrophysiological parameters are independent of learning effects or psychological biases, are safe and methodically simple, and can easily be implemented in the current fatigue diagnostics. Especially from a clinical perspective, the parameters complement the present fatigue concept and help to promote a broader recognition of fatigue and fatigability in clinical subgroups. Finally, anodal tDCS over the left dorsolateral prefrontal cortex can counteract fatigability, indicating its therapeutic potential for treating fatigability in neuropsychiatric diseases.

# 3.4 Fatigability-related oscillatory brain activity changes in people with MS

*The content of this chapter is under review as: Linnhoff, S., Haghikia, A., & Zaehle, T. Fatigability-related oscillatory brain activity changes in people with MS.*

## 3.4.1 Abstract

*Background:* Fatigue, a multidimensional and challenging symptom associated with various underlying conditions, can manifest as a subjective feeling and a performance fatigability. The latter is often defined as an objectively measurable performance decline with time on task. Both syndromes are highly prevalent in people with multiple sclerosis (pwMS) and are often resistant to medical therapy. In the absence of valid and reliable objective parameters, the current fatigue diagnosis remains purely subjective. Assessing brain wave activity changes has repeatedly been a viable strategy to monitor mental fatigue in healthy subjects. In this study, we aimed to investigate oscillatory brain activity changes and their associations with subjective fatigue in pwMS.

*Methods:* We enrolled 21 pwMS and 21 healthy controls (HC) in this study. They performed a 30-minute cognitively exhaustive task and were repeatedly asked about their subjective feelings. Resting-state EEGs were performed before and after the task.

*Results:* Our results revealed a systematically stronger fatigability development in pwMS that was objectively measurable. PwMS reported lower mental fitness levels and demonstrated greater variability in reaction times with time on task. Occipital alpha power significantly increased during the task. Especially for upper alpha power, this increase was significantly more prominent in pwMS compared to HC. However, the time-on-task-induced changes in our study were not associated with the subjective fatigue ratings.

*Conclusions:* The results of this study help improve the understanding of the neural mechanisms underlining cognitive fatigability and may complement the fatigue diagnostis and therapy monitoring with quantitative objective methods.

## 3.4.2 Introduction

Fatigue affects a large proportion of people with multiple sclerosis (pwMS) and often restricts their life already at the earliest stages of the disease (van der Vuurst de Vries et al., 2018). It dramatically worsens the quality of life in pwMS and is the leading cause of early retirement (Kobelt et al., 2017; Krause et al., 2013). Yet, to date, the pathophysiological mechanisms underlying MS-related fatigue are still unclear, and disease specific therapy is lacking.

From a clinical point of view, as is also reflected in the fatigue definition of the MS council, fatigue is a subjective symptom "that is perceived by the individual" (Multiple Sclerosis Council for Clinical Practice Guidelines, 1998, p. 2). Therefore, the current fatigue diagnosis is mainly based on subjective questionnaires. Those self-reports, however, are of retrospective nature and, therefore, mood-sensitive and subject to psychological biases. Additionally, many items coincide with items regarding symptoms of depression, making differentiation difficult (Bol et al., 2009). Thus, for a better pathophysiological understanding and treatment evaluation of MS-related fatigue, it is of utmost importance to expand the fatigue diagnostics with the objective assessment of its impact on patients´ daily performance. So far available studies report little to no association between the perceived feeling of fatigue and an objectively measurable performance decline (Linnhoff et al., 2019). Therefore, the current understanding of the fatigue concept includes both components as distinct symptoms. Fatigue, the subjective feeling of exhaustion, is referred to as the trait component. And fatigability, the inability to sustain mental performance over an extended period of time, the state component (Kluger et al., 2013; Linnhoff et al., 2019). Both can either occur simultaneously or distinct from another in pwMS (Enoka et al., 2021; Hanken et al., 2014).

State fatigue can be assessed either subjectively via visual analog scales (VAS) or objectively via performance changes with time on task. There has been a variety of objective parameters that have been examined in previous studies with inconsistent results. Thus, despite subjective reports of high levels of fatigue, pwMS are often able to maintain their behavioral performance when measured with reaction times and accuracy (Linnhoff et al., 2019). As a result, finer-grained behavioral analyses have been proposed, such as the analysis of reaction time variability (Bodling et al., 2012; Bruce et al., 2010).

Other neuropsychological assessments that have been proposed, like the Paced Auditory Serial Addition Test (PASAT), are susceptible to learning strategies and aversive for the participants (Agyemang et al., 2021).

Electrophysiological parameters have the advantage that they are not subject to psychological biases or subjective manipulation. Additionally, they give further insights into the neuronal alterations underlying fatigue and fatigability. In healthy subjects, fatigability has repeatedly been associated with increased frontomedial theta (fm-theta) as well as occipital alpha power (Boksem et al., 2005; Craig et al., 2012; Linnhoff et al., 2021; Tran et al., 2020; Wascher et al., 2014). Thus, an increase in alpha power was observed during resting state EEGs (Barry et al., 2007) or along with increasing error rates and reaction times during exhausting tasks (Gharagozlou et al., 2015; Wascher et al., 2014). According to the oscillatory model of sustained attention by Clayton et al. (2015), fatigability results in a systematic shift from fast to low-frequency waves. Fm-theta power increases as a result of compensatory mechanisms to improve top-down control processes, whereas alpha power increases over task-relevant cortical areas suppressing information processing and resulting in attention deficits.

Finally, a large number of imaging studies demonstrated relations between MS-related fatigue and structural and functional abnormalities in the cortico-striato-thalamo-cortical network, the fatigue network (Ayache & Chalah, 2017). In particular, frontal activity changes have often been associated with increased subjective trait fatigue (Ayache & Chalah, 2017; Barbi et al., 2022). Frontally modulated compensatory mechanisms, such as increased fm-theta acitivity, might therefore be disturbed in pwMS, resulting in a stronger increase of occipital alpha power. Thus, the present study aimed to investigate oscillatory brain wave activity changes with time on task in pwMS and healthy controls (HC). We hypothesized that pwMS, compared to HC, will experience greater fatigability with time on task. This will lead to a more significant increase in subjective ratings as well as objectively measurable differences in reaction time variability and oscillatory brain wave activity.

## 3.4.3 Methods

### 3.4.3.1 Participants

We enrolled 21 pwMS and 21 HC (see Table 6 for demographic and clinical characteristics). Inclusion criteria for pwMS were a minimum of three months since the last relapse or use of corticosteroids, no color blindness, no current neurological or psychiatric comorbidities, and no current treatment with fatigue or antidepressant medication. All pwMS were diagnosed with clinically definite MS according to the McDonald criteria and were recruited from the outpatient pool of the University Hospital of Magdeburg. Nineteen subjects had a relapsing-remitting course of MS, one a primary progressive, and one a secondary progressive form. Disease-modifying therapy (DMT) consisted of Glatirameracetat ($n$ = 5), Natalizumab ($n$ = 3), Siponimod ($n$ = 1), Fingolimod ($n$ = 5), Dimethylfumarat ($n$ = 1), Interferon-Beta ($n$ = 1), Ocrelizumab ($n$ = 2), and Cladribin ($n$ = 1). Two subjects received no DMT. Inclusion criteria for HC were no history of neurological or psychiatric disorders, no color blindness, and no current depression (Beck Depression Inventory II - Fast Screen, BDI-FS ≤ 4) or sleep disorder (Epworth Sleepiness Scale, ESS ≤ 10). The local ethic committee of the University of Magdeburg approved the study. All subjects provided written consent according to the Declaration of Helsinki and received a monetary reward (Euro 30 in total).

**Table 6.** *Baseline group characteristics, mean (± SD).*

| | **pwMS** | **HC** | |
|---|---|---|---|
| gender f/m | 15/6 | 14/7 | |
| age [years] | 42.29 (12.81) | 40.48 (13.21) | $p$ = .597 |
| BDI-FS [points] | 2.43 (2.80) | 1.29 (1.10) | $p$ = .161 |
| ESS [points] | 10.86 (3.49) | 5.71 (2.67) | $p$ < .001 |
| SDMT [points] | 59.91 (10.07) | 63.22 (8.68) | $p$ = .262 |
| $WEIMuS_{total}$ [points] | 35.10 (15.50) | - | |
| $WEIMuS_{cognitive}$ [points] | 17.76 (7.75) | - | |
| disease duration [years] | 11.90 (9.29) | - | |
| EDSS [points] | 2.67 (1.50) | - | |

*BDI-FS,* Becks Depression Inventory – Fast Screen; *EDSS,* Expanded Disability Status Scale; *ESS,* Epworth Sleepiness Scale; *HC,* healthy controls; *MS,* Multiple Sclerosis; *WEIMuS,* Wuerzburg Fatigue Inventory for Multiple Sclerosis

### 3.4.3.2 Procedure

All subjects signed informed consent and completed several questionnaires for handedness (Edinburgh Handedness Inventory), current mood (BDI-FS), and daytime sleepiness (ESS). PwMS additionally completed the Wuerzburg Fatigue Inventory (WEIMuS) to assess their subjective trait fatigue. All subjects performed Ishihara´s Test for color blindness and the Symbol Digit Modalities Test (SDMT) to evaluate cognitive functioning. After electroencephalogram (EEG) mounting, the subjects received instruction for the following task and performed one training block consisting of 20 trials. The study then started with the presentation of electrical visual analog scales (VAS) from 0 to 100. To systematically investigate subjective state ratings, we used three different VAS scales. One was positively phrased, asking the subjects "how mentally fit" ($VAS_{fit}$) they felt "right now at this moment", one rather negatively phrased, asking "how mentally exhausted" ($VAS_{ex}$) they felt "right now at this moment", and the third asked about "how much their mind has wandered" ($VAS_{mind}$) during the last block. Before starting the task, only $VAS_{fit}$ and $VAS_{ex}$ were presented, as no mind wandering could have occurred then. Thereafter, an 8-minute resting-state EEG was recorded consisting of eight alternating one-minute blocks of eyes-open and eyes-closed (see Figure 13 for an illustration of the study design).

The fatigability-inducing task (a continuous performance task, CPT) was adapted from Wascher et al. (2014). Subjects performed six blocks (B1-B6) á 110 trials (duration approximately 5 minutes per block). Every trial consisted of two sequentially presented frames. The first frame presented two gray bars left and right to a fixation cross. It was presented for 200 ms followed by a 50 ms blank interval and then the second frame for 200 ms. In the second frame, one of the two bars changed its color to red or blue. The subjects were asked to indicate which color change occurred (the right Ctrl key for red and the left Ctrl key for blue). A 90-second break separated every block. Every second block, the three VAS scales were presented. Directly after the CPT task, a second resting-state EEG was recorded.

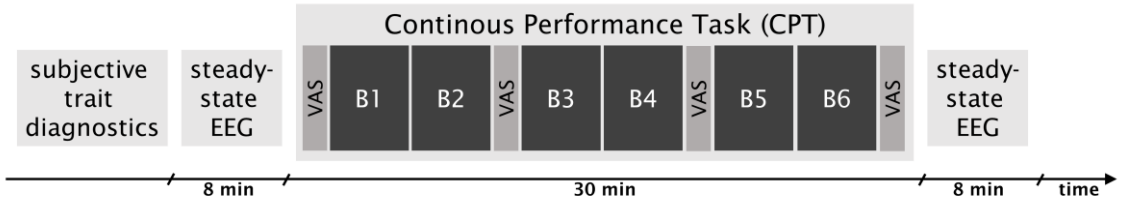


**Figure 13.** Experimental design. After assessing demographic and clinical data via self-report questionnaires, an 8-minute resting state EEG with alternating eyes-open and eyes-closed segments was performed. A 30-minute continuous performance task (CPT) followed that consisted of six blocks (B1-B6) of 5 minutes each. The first resting state EEG and B1 of the CPT task were used as baseline measures. Before the first and after each second block, subjects were asked about their current perceived fatigue status on visual analog scales (VAS). Subsequently, a second resting state EEG was performed.

### 3.4.3.3 EEG signal recording and preprocessing

EEG was recorded at Fp1, Fp2, F3, Fz, F4, FCz, C3, Cz, C4, P3, Pz, P4, POz, O1, Oz, and O2 using Ag/AgCl-electrodes mounted in an elastic cap (EasyCap GmbH, Germany). The ground electrode was attached to the AFz position, and all channels were referenced to the left and right mastoid. Additionally, an electrooculogram (EOG) was recorded. The data was recorded by Brain DC amplifier (Brain Products, Germany) sampled at 1000 Hz. Impedances were kept below 5 kΩ. EEG preprocessing and data analysis were carried out in BrainVision Analyzer 2.1 (Brain Products, Germany).

The EEG data were resampled to 512 Hz, band-pass filtered from 0.1 to 40 Hz, and then corrected for eye-movement artifacts using the Gratton and Cole method (Gratton et al., 1983). The data were then further analyzed separately for the pre and post resting state EEG segments and the six task blocks. Subsequently, 2 s long segments with an overlap of 200 ms were extracted from the continuous EEG and submitted to a fast Fourier transformation, using a Hanning window with 10% of the total segment length. After averaging, spectral power was extracted for the theta (4.5 to 6 Hz), lower alpha (8 to 9.5 Hz), and upper alpha band (10 to 12.5 Hz) by averaging power values across respective 1-Hz bins. We conducted two regions of interest, the mid-frontal region

(Fz, FCz, and Cz) to assess fm-theta power and the occipital region (POz and Oz) to assess occipital alpha power.

#### 3.4.3.4 Statistical analysis

R Statistical Software (version 4.2.0, R Core Team, 2022) and JASP software (version 0.16.3, JASP Team, 2022) were used for statistical analyses and production of all plots.

For the analysis of fatigability-induced changes during the resting-state EEGs, we analyzed spectral changes from the eyes-open condition of the resting state EEG data and performed 2 x 2 repeated measures of analysis of variance (ANOVAs) with the within-subject factor time (pre, post) and the between-subject factor group (pwMS, HC). The power data were log-transformed, as fm-theta and alpha power tended to be skewed.

To investigate the effects of time on task, we analyzed subjective (VAS scores), behavioral (reaction time variability, RT variability), and electrophysiological (lower, upper alpha, and fm-theta power) fatigability values. The data was analyzed using (General) Linear Mixed Models [(G)LMMs]. The subjective and behavioral data were normally distributed, whereas the band power values were log-distributed. Thus, LMMs using the *lmer* function and GLMMs using the glmer function, with gaussian log family, both from the *afex* (Singmann et al., 2022b) package, were performed. P values were obtained using Sattersthwaite´s approximation method for LMMs and Wald Chisquare Tests from the car (Fox & Weisberg, 2019) package or GLMMs. We excluded invalid and error trials as well as physiologically unreasonable reaction times below 200 ms from the RT data analysis. Furthermore, for all data analyses, outliers below or above 1.5 times the interquartile range were identified and adjusted to this limit to reduce the impact of outliers without having to remove them. Subjective data, RT variability, and band power data were considered as dependent variables. Time, group, and group x time were considered as fixed factors. Data from HC in block B1 were used as baseline. Individuals and their variation of the dependent variable over time were used as random effects.

## 3.4.4 Results

### 3.4.4.1 Fatigability-related activity changes with time on task

The model to predict $VAS_{fit}$ ratings showed a significant effect of *time* [$F(1,40) = 74.993$, $p < .001$, $\eta_p^2 = .65$] as well as an interaction between *time* and *group* [$F(1,40) = 5.767$, $p = .021$, $\eta_p^2 = .13$]. The main effect *group* was not significant [$F(1,40) = 2.301$, $p = .137$]. The initial $VAS_{fit}$ ratings for the HC group were 80.97 points ($\beta_{intercept}$, $t(40) = 25.787$, $p < .001$) and 74.24 points ($\beta_{intercept} + \beta_{group}$) for pwMS (see Table 7). With each new query, the ratings decreased by 7.50 points ($\beta_{time}$, $t(40) = -4.425$, $p < .001$) in HC, while they decreased by 13.27 points ($\beta_{time} + \beta_{time*group}$) in pwMS (see Figure 14A). The model explained approximately 64 % of the variance (fixed and random effects, $R^2 = 0.639$).

Contrary, the models to predict $VAS_{ex}$ as well as $VAS_{mind}$ ratings showed a significant effect of *time* [$VAS_{ex}$: $F(1,40) = 50.081$, $p < .001$, $\eta_p^2 = .56$; $VAS_{mind}$: $F(1,40) = 15.070$, $p < .001$, $\eta_p^2 = .27$], but no significant *time* x *group* interaction [$VAS_{ex}$: $F(1,40) = 0.038$, $p = .846$; $VAS_{mind}$: $F(1,40) = 0.123$, $p = .728$] (see Figure 2B + 2C). Additionally, there was a significant difference of initial $VAS_{ex}$ ratings [$F(1,40) = 17.689$, $p < .001$, $\eta_p^2 = .31$], with the MS group having higher initial ratings by 20 points ($\beta_{group} = 20.226$, $t(40) = 4.206$, $p < .001$, see Table 2).

**Table 7.** *β-coefficients of (G)LMMs.*

| | | *β* | SE *β* | *t*-value | *p*-value |
|---|---|---|---|---|---|
| VAS mental fitness [points] | | | | | |
| | intercept | 80.971 | 3.140 | 25.787 | < .001 |
| | time | -7.505 | 1.696 | -4.425 | < .001 |
| | group | -6.736 | 4.441 | -1.517 | .137 |
| | time * group | -5.760 | 2.398 | -2.401 | .021 |
| VAS mental exhaustion [points] | | | | | |
| | intercept | 13.714 | 3.401 | 3.887 | < .001 |
| | time | 9.502 | 1.848 | 5.143 | < .001 |
| | group | 20.226 | 4.809 | 4.206 | < .001 |
| | time * group | -0.512 | 2.613 | -0.196 | .846 |
| VAS mind wandering [points] | | | | | |
| | intercept | 24.095 | 5.928 | 4.064 | < .001 |
| | time | 5.405 | 2.164 | 2.497 | .017 |
| | group | 9.873 | 8.384 | 1.178 | .246 |
| | time * group | 1.071 | 3.061 | 0.350 | .728 |
| RT variability [ms] | | | | | |
| | intercept | 82.324 | 5.996 | 13.731 | < .001 |
| | time | 1.026 | 1.383 | 0.742 | .462 |
| | group | -4.639 | 8.479 | -0.547 | .587 |
| | time * group | 5.147 | 1.955 | 2.632 | .012 |
| fm-theta power [log] | | | | | |
| | intercept | 1.196 | 0.061 | 19.632 | < .001 |
| | time | 0.014 | 0.009 | 1.487 | .137 |
| | group | -0.096 | 0.087 | -1.106 | .269 |
| | time * group | -0.002 | 0.013 | -0.155 | .877 |
| lower alpha power [log] | | | | | |
| | intercept | 0.632 | 0.136 | 4.644 | < .001 |
| | time | 0.066 | 0.016 | 4.072 | < .001 |
| | group | 0.259 | 0.191 | 1.358 | .174 |
| | time * group | -0.008 | 0.022 | -0.352 | .725 |
| upper alpha power [log] | | | | | |
| | intercept | 0.876 | 0.115 | 7.640 | < .001 |
| | time | 0.023 | 0.013 | 1.950 | .051 |
| | group | 0.158 | 0.161 | 0.977 | .329 |
| | time * group | 0.035 | 0.018 | 1.973 | .049 |

*fm,* fronto-medial; *log,* log-transformed; *RT,* reaction time; *SE,* standard error; *VAS,* visual analog scale

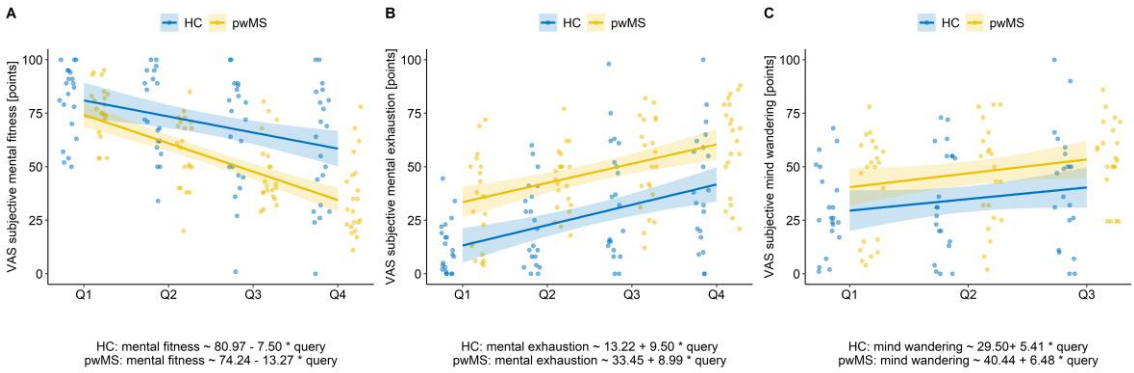


**Figure 14.** Results of the linear mixed model to predict subjective state ratings: Regression plots representing perceived mental fitness (A), mental exhaustion (B), and mind wandering (C) against the number of queries (Q1-Q4) separate for the HC and pwMS groups.

The model to predict RT variability showed a significant main effect of *time* [$F(1,40) = 13.554$, $p < .001$, $\eta_p^2 = .25$] and interaction effect between *time* and *group* [$F(1,40) = 6.929$, $p = .012$, $\eta_p^2 = .15$] but no significant effect of *group* [$F(1,40) = 0.004$, $p = .952$]. Thus, initial RT variability did not vary between HC and pwMS ($\beta_{group} = -4.639$, $t(40) = -0.547$, $p = .587$) and the increase of RT variability with with time on task in HC of 1.02 ms per block was not significant [$\beta_{time} = 1.026$, $t(40) = 0.742$, $p = .462$]. Contrary, in pwMS, RT variability significantly increased by 6.17 points ($\beta_{time} + \beta_{time*group}$) per block ($\beta_{time*group} = 5.147$, $t(40) = 2.632$, $p = .012$) (see Figure 3). The model explained approximately 65 % of the variance (fixed and random effects, $R^2 = 0.653$).

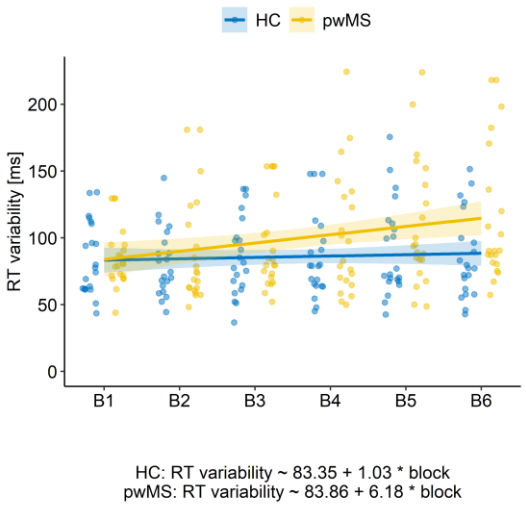


**Figure 15.** Results of the linear mixed model to predict reaction time (RT) variability ratings: Regression plots representing RT variability against time on task (block B1-B6) separate for the HC and pwMS groups.

The GLMM to analyze fm-theta power showed a marginally significant effect of *time* [$\chi^2(1) = 3.652$, $p = .056$] but no significant effect of *group* [$\chi^2(1) = 1.372$, $p = .241$] and no significant interaction of *time* and *group* [$\chi^2(1) = 0.024$, $p = .877$]. Thus, there was a trend of fm-theta power increasing with time on task, but this was unaffected by group. Fixed and random effects explained approximately 34 % of the variance ($R^2 = 0.338$). The model predictions, thus, the estimated $\beta$-values that describe the parameters as a function of time and group are shown in Table 7.

Analyzing occipital lower alpha power showed a significant effect of *time* [$\chi^2(1) = 32.265$, $p < .001$] but no significant *group* effect [$\chi^2(1) = 1.747$, $p = .186$] and no interaction of *time* and *group* [$\chi^2(1) = 0.124$, $p = .725$]. Lower alpha power significantly increased by 1.09 µV² (SD = 1.30 µV²) in HC and by 1.62 µV² (SD = 2.45 µV²) in pwMS. The model explained approximately 49 % of the variance (fixed and random effects, $R^2 = 0.493$). The GLMM to analyze upper alpha power revealed a significant effect of *time* [$\chi^2(1) = 27.383$, $p < .001$] and a significant interaction of *time* and *group* [$\chi^2(1) = 3.894$, $p = .048$]. The main effect *group* was not significant [$\chi^2(1) = 1.921$, $p = .166$]. Thus, while both groups did not differ at the beginning of the task, upper alpha

power more strongly increased with time on task in pwMS (MW = 1.62 µV², SD = 2.12 µV²) than in HC (MW = 0.52 µV², SD = 0.71 µV²). Fixed and random effects explained approximately 45 % of the variance ($R^2$ = 0.455). The model predictions, thus, the estimated $\beta$-values that describe the parameters as a function of time and group are shown in Table 7.

The linear regression plots of the non-transformed data of fm-theta and alpha (low and upper) power are shown in Figure 16A-C, and the β-coefficients representing the fixed effects are listed in Table 7. However, keep in mind that the data was analyzed using GLMMs with log link function. Hence, the predicted β-coefficients are log-transformed and not applicable to the figure.

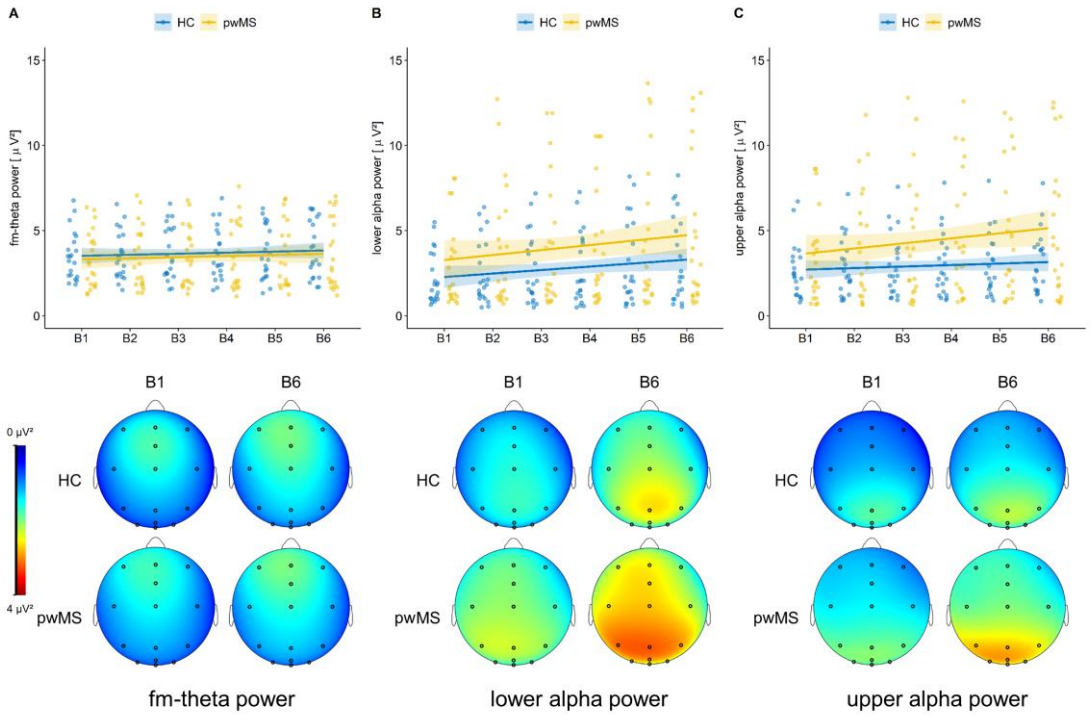


**Figure 16.** Regression plots representing fm-theta power (A, top), occipital lower alpha power (B, top), and occipital upper alpha power (C, top) against time on task (block B1-B6) separate for the HC and pwMS groups. The bottom row represents fm-theta (A, bottom), occipital lower alpha (B, bottom), and occipital upper alpha (C, bottom) topography plots in B1 and B6 for the HC and pwMS groups.

Finally, we investigated the general interrelationship between fatigue self-reports (WEIMuS cognitive scores and delta scores of subjective ratings on mental fitness) in pwMS and those objective parameters that indicated a different time on task dynamic of pwMS and HC (delta scores of RT variability and upper alpha power (log-transformed)). However, the data showed no significant relationship between the changes in both objective parameters and subjective trait fatigue (all *ps* > .102).

#### 3.4.4.2 Fatigability-related activity changes during resting-state EEGs

The pre vs. post analyses of the resting EEG data confirmed the task-related results. Thus, the upper alpha power showed no effect of *group* [$F(1,40) = 0.315$, $p = .578$] but a significant main effect of *time* [$F(1,40) = 19.772$, $p < .001$, $\eta_p^2 = .33$] with the power increase being more prominent in pwMS. However, the interaction was not significant [$F(1,40) = 2.138$, $p = .151$]. Furthermore, for the fm-theta power analyses, the ANOVA revealed no main effects of *time* [$F(1,40) = 2.472$, $p = .124$] and *group* [$F(1,40) = 1.185$, $p = .283$] and no significant interaction [$F(1,40) = 0.080$, $p = .778$]. Similarly, for lower alpha power, we found a significant main effect of *time* [$F(1,40) = 14.497$, $p < .001$, $\eta_p^2 = .27$] but no *group* effect [$F(1,40) = 0.262$, $p = .612$] and no interaction [$F(1,40) = 0.139$, $p = .711$]. The log-transformed power values as a function of time separate for both groups are shown in Figure 17A-C.

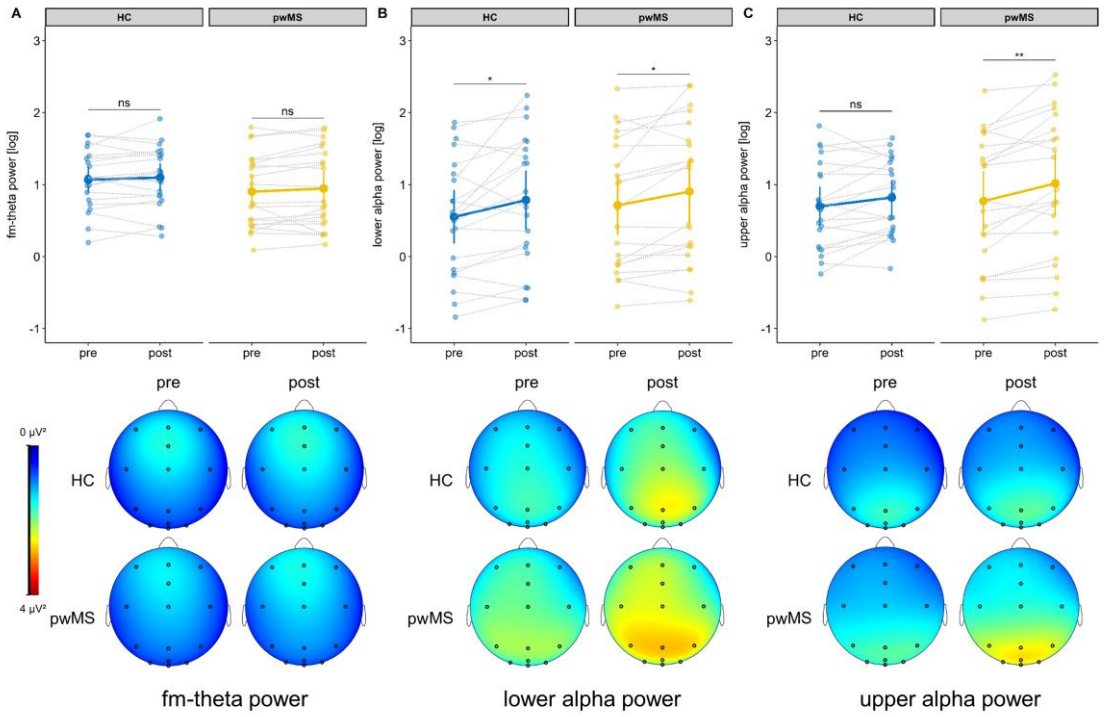


**Figure 17.** Regression plots representing fm-theta power (A, top), occipital lower alpha power (B, top), and occipital upper alpha power (C, top) against resting state EEG session (pre, post) separate for the HC and pwMS groups. The bottom row represents fm-theta (A, bottom), occipital lower alpha (B, bottom), and occipital upper alpha (C, bottom) topography plots in pre and post session for the HC and pwMS groups.

### 3.4.5 Discussion

This study systematically investigated fatigability-related spectral power changes in pwMS and HC. As hypothesized, pwMS experienced a greater fatigability with time on task compared to HC. They felt significantly less mentally fit, and, in the objective parameters, they showed greater variability in RT and increased occipital upper alpha power with time on task. Comparable results were shown in resting state EEG data. The changes in the objective parameters, however, were not associated with the changes in subjective state rating as well as with the trait fatigue scores.

#### 3.4.5.1 Oscillatory changes

Our results revealed an increase in occipital alpha power in pwMS. Especially for upper alpha power, this increase was significantly more prominent in pwMS compared to HC. This, together with the subjective and behavioral data, supports the assumption of a more severe fatigability in pwMS suffering from trait fatigue. Thus, while our results revealed no initial differences in band power values in pwMS and HC, pwMS showed a systematically more severe and faster fatigability that was objectively measurable.

An increase in occipital alpha power during sustained attention tasks is generally in line with previous literature (Boksem et al., 2005; Clayton et al., 2015; Craig et al., 2012; Gharagozlou et al., 2015). Alpha oscillations have consistently been associated with the suppression of distracting information by inhibiting sensory modalities irrelevant to the task. Therefore, alpha power may play a pivotal role in fatigability development, impairing the attentional focus when increasing over task-relevant areas, such as the occipital cortex in a visual attention task (Clayton et al., 2015). However, other studies report a controversial alpha power decrease with time on task (Ishii et al., 2013; Klimesch, 1999; Li et al., 2020). However, there are significant differences between the assessment methods, tasks, and also the duration of the tasks, so comparing the results is difficult. Additionally, all of the studies examined young, healthy subjects and no clinical subgroups. Nevertheless, more research is needed to use alpha power as a diagnostical marker for fatigability in pwMS.

On the contrary, we did not find an fm-theta power increase as reported in the previous literature (Boksem et al., 2005; Clayton et al., 2015; Craig et al., 2012; Wascher et al., 2014). For HC, this may result from a lower level of fatigability. Thus, HC in our study remained mentally fit and were able to uphold their behavioral performance. In addition, Wascher et al. (2014) reported that fm-theta power increased steadily over the course of four hours on the task. Similarly, other studies have examined theta increases in healthy subjects over more extended periods of time than 30 minutes (Linnhoff et al., 2021; Tran et al., 2020). However, as the task was already very exhausting for pwMS, we decided not to extend it further. Contrary, in pwMS, who were demonstrably fatigued during our task, the lack of fm-theta power increase could be related to the malfunctioning cortico-striato-thalamo-cortical network that has been proposed in previous studies

(Ayache & Chalah, 2017). In this way, results from several neuroimaging studies demonstrated relations between subjective trait fatigue and structural as well as functional abnormalities in different cortical regions, including the frontal cortex (Pardini et al., 2010; Roelcke et al., 1997; Sepulcre et al., 2009). According to Clayton's model of sustained attention (Clayton et al., 2015), this underactivity of the frontal cortex in fatigued pwMS might lead to the lack of compensatory fm-theta power mechanisms and, thus, to disturbed top-down control processes.

Our findings give important new insights into fatigability-related oscillatory activity changes. Furthermore, they may help to extend the therapeutic options for pwMS. As such, transcranial electrical stimulation (tES) may provide the unique opportunity to manipulate this maladaptive neural activity underlying fatigability. In our recent study, we already demonstrated that transcranial direct current stimulation (tDCS) counteracted fatigability development in healthy subjects and reduced the increase of occipital alpha power (Linnhoff et al., 2021). Future studies might use transcranial alternating current stimulation (tACS) to stimulate targeted frequencies selectively and investigate the causal role of oscillational activity in a fatiguing brain. By using tACS in the gamma range while performing a vigilance task, Loeffler et al. (2018) aimed to decrease inhibitory alpha power in task-relevant cortical areas. Gamma tACS counteracted the increase in reaction times with time on task. However, the effects on occipital alpha power remain to be determined due to missing EEG recordings.

#### 3.4.5.2 Subjective assessment of fatigability

In this study, we systematically investigated subjective state fatigue ratings with time on task via three different VAS scales. PwMS felt significantly less fit with time on task compared to HC. On the contrary, ratings on mental exhaustion as well as mind wandering increased similarly in both groups, while pwMS reported higher exhaustion ratings at baseline.

Our results demonstrate how differently fatigue can be perceived and how important it is for future studies to pay attention to how scales are phrased. In general, self-reports are subject to psychological errors and strongly depend on individual trait complexes (Ackerman & Kanfer, 2009). PwMS are frequently asked about their current level of exhaustion during clinical exams, which increases their individual awareness of

the syndrome. Consequently, especially for mental exhaustion, the higher baseline ratings may result from priming. Furthermore, our results confirm the complex relationship between subjective fatigue ratings and objectively measurable fatigability parameters. In this study, we did not find associations between changes in subjective ratings and the changes in the objective parameters. Thus, as demonstrated in previous studies, our results support the assumption that trait and state fatigue, as well as fatigability parameters, might be independent dimensions of an overall MS-related fatigue that may either jointly appear or occur independently of one another (Enoka et al., 2021; Hanken et al., 2014). In future studies, it might be helpful to use questionnaires that primarily assess fatigability, such as the Pittsburgh Fatigability Scale (PFS) (Glynn et al., 2015; Renner et al., 2021). It measures perceived mental fatigability in the daily life and might be a more suitable subjective marker for a correlation with the cognitive decline with time on task. In general, our findings demonstrate the importance of incorporating subjective and objective fatigue in clinical fatigue diagnostic and research. Future studies need to investigate fatigue as a holistic syndrome with fatigability being a part of it and need to pay attention to a unified fatigue taxonomy.

#### 3.4.5.3 Limitations

Our study is not without limitations. First, the different VAS scales were always presented in the same order, which may have led to order effects. Additionally, the repetitive assessment of perceived feelings might have resulted in socially desirable answers and increased self-awareness. Second, we did not include a sample with MS but without fatigue. Thus, one may argue that our results can be attributed to MS instead of MS-related fatigue. It should be noted, however, that we did not observe baseline differences between both groups. Additionally, the SDMT scores, which indicate cognitive functioning, neither revealed differences. Thus, we argue that our groups did not differ according to their overall cognitive ability but rather in their cognitive decline resulting from fatigability development with time on task. Finally, pwMS with relapsing-remitting MS form are overrepresented in our study, indicating that the effects may be specific for this subtype. Excluding both pwMS with primary- and secondary-progressive MS form did not change the results. Future studies should consider this and possibly investigate subtypical effects in more detail. However, it should be noted that the distribution of MS forms in our sample is comparable to the general distribution among pwMS.

#### 3.4.5.4 Conclusion

In summary, our results demonstrated a stronger fatigability development in pwMS compared to HC. PwMS reported a more prominent decrease in mental fitness ratings. Importantly, this systematic increase in fatigability was objectively measurable. Compared to HC, pwMS showed a stronger increase in RT variability as well as an enhanced increase in occipital upper alpha power. To our knowledge, this is the first study providing evidence for specific fatigability-related brain wave activity changes in pwMS. Our results provide new insights and help to improve the understanding of fatigability-related pathomechanisms in pwMS as well as healthy subjects.

# 3.5 Cognitive fatigue-related sensory gating deficits in people with MS

*The content of this chapter is under review as: Linnhoff, S., Haghikia, A., & Zaehle, T. Fatigue-related sensory gating deficits in people with multiple sclerosis: a case-control study.*

## 3.5.1 Abstract

*Background and Objectives:* Cognitive fatigue is highly prevalent in people with multiple sclerosis (pwMS) and significantly limits their quality of life. Fatigue can be subdivided into a subjective feeling of constant (trait) or current (state) exhaustion, as well as an objective performance decline, also known as fatigability. However, the current fatigue diagnosis in pwMS is purely subjective, leaving fatigability mostly unattended. Sensorimotor and sensory gating deficits have recently been described as possible objective markers for fatigability in healthy subjects. Thus, this study aimed to investigate the potential of prepulse inhibition (PPI) ratios and the P50 sensory gating suppression as surrogate markers for cognitive fatigue in pwMS.

*Methods:* PPI and P50 sensory gating ratios were assessed before and after a 30-minute fatigability-inducing AX- continuous performance task. Subjective trait fatigue was operationalized via self-report questionnaires, subjective state fatigue via visual analog scales (VAS), and fatigability via the change in both gating ratios. The data were analyzed using Linear Mixed Models and Pearson correlations.

*Results:* We included 18 pwMS and 20 healthy controls (HC) in the final analyses. The task-induced fatigability was more pronounced in pwMS. While the initial PPI and P50 ratios were similar in both groups, P50 sensory gating was significantly disrupted after fatigability induction in pwMS. PPI, on the other hand, decreased in both groups. Moreover, initial P50 sensory gating ratios were negatively associated with subjective trait fatigue in pwMS, indicating that higher trait fatigue is associated with disrupted sensory gating. Finally, fatigability-related changes in P50 sensory gating were associated with the changes in VAS ratings, but only in HC.

*Discussion:* This study demonstrated that P50 sensory gating is a promising objective fatigue and fatigability parameter. Importantly, P50 sensory gating correlated with subjective trait and state fatigue ratings. Our results extend the subjective fatigue diagnosis and broaden the understanding of pathophysiological neuronal mechanisms in MS-related fatigue. This is the first study to present fatigue-related disruption of sensory gating in pwMS.

## 3.5.2 Introduction

Cognitive fatigue in people with MS (pwMS) is defined as „a subjective lack of […] mental energy that is perceived by the individual or caregiver to interfere with usual and desired activities“ (Multiple Sclerosis Council for Clinical Practice Guidelines, 1998, p. 2). It affects up to 80 % of pwMS (Cook et al., 2013) and is associated with a decreased quality of life (Kobelt et al., 2017; Yamout et al., 2013). Fatigue is currently understood as a subjective syndrome with a trait characteristic. In contrast, the inability to maintain a certain performance level over a sustained period of cognitive effort is defined as fatigability (Holtzer et al., 2011; Kluger et al., 2013). Thus, fatigability is, per definition state-dependent and can be assessed in clinical subgroups, like pwMS, but also in healthy subjects. In pwMS, Dettmers et al. (2021) recently reported that fatigability rather than fatigue predicts the employment status in pwMS. Accordingly, we and others proposed a unified fatigue taxonomy according to which cognitive fatigue in pwMS can be subdivided into subjective and objective fatigue (Kluger et al., 2013; Linnhoff et al., 2019). Subjective fatigue has a trait and state characteristic. It can be assessed via self-report questionnaires (trait) or visual analog scales (state). Objective fatigue, or fatigability, however, has only a state component and can be assessed via the change in an objective measure over a sustained period of time (Linnhoff et al., 2019). The current fatigue diagnostic purely focuses on subjective trait fatigue, leaving subjective state fatigue but also fatigability unattended. However, complementing the current fatigue diagnostic with objectively measurable parameters is of utmost importance. Some objective parameters for assessing fatigability have been presented in the literature but with varying results. Behavioral parameters, such as reaction time or accuracy, tend to be susceptible to learning effects. Additionally, very few studies find a relationship between subjective and objective fatigue and fatigability (see Linnhoff et al., 2019 for a comprehensive review).

To date, there is no consensus on the neurological pathomechanisms responsible for the development of the fatigue syndrome or its variability over time. Recently, several studies highlighted the important role of the thalamus for MS-related fatigue (Barbi et al., 2022; Capone et al., 2020). These studies report altered thalamus activity at resting in pwMS with trait fatigue but also during exhaustive tasks. The thalamus serves as a pivotal hub in several cognitive processes. Therefore, even small changes in the thalamus activity might substantially impact the complex brain system, leading to objectively measurable differences in cognitive processes related to the thalamus. One of those processes that plays an important role in cognitive top-down control and is processed by the thalamus, is sensory gating (Bak et al., 2014; Conte et al., 2020). By filtering out redundant or irrelevant information, it serves as an involuntary and preconscious mechanism to protect stimulus processing. Thus, it can be quantified via the percentage of prepulse inhibition (PPI) or the suppression of the P50 event-related potential. In a typical PPI paradigm, an intense stimulus is presented that produces a muscular startle reflex. This muscular reflex is reduced (inhibited) if a stimulus of lower intensity (prepulse) was previously presented. Similarly, the P50 ERP typically evoked using the auditory paired click paradigm is reduced after the second click tone. Accordingly, in both paradigms, the processing of the second stimulus is suppressed by the processing of the first stimulus, leading to a quantifiable reduction of reflex or amplitude to the second stimulus. Gating, in general, is a protective mechanism of the cortex to prevent the brain from overstimulation, allowing for coherent thought. Accordingly, it is likely to assume that deficits in gating may result in the misinterpretation of sensory information that would subsequently lead to fatigue. This assumption has already been confirmed by studies investigating healthy subjects, which reported disrupted gating after cognitive (Linnhoff et al., 2021; van der Linden et al., 2006) or physical exhaustion (Aleksandrov et al., 2016). Additionally, sensory gating has already been reported as a reliable surrogate marker for various attention-related diseases such as schizophrenia (Shen et al., 2020; Xia et al., 2020) and attentional-deficit hyperactivity disorder (Holstein et al., 2013; Micoulaud-Franchi et al., 2019). In our recent study, we could also show that transcranial direct current stimulation (tDCS) over the left dorsolateral prefrontal cortex counteracted fatigability development and reduced the gating deficits in healthy subjects (Linnhoff et al., 2021).

To our knowledge, no study has investigated sensorimotor and sensory gating deficits as a result of cognitive fatigue and fatigability in pwMS. Thus, the present study

aims to develop a deeper understanding of the pathophysiological processes of MS-related fatigue and fatigability and to complement the purely subjective fatigue diagnostic in pwMS with objective fatigability parameters. We hypothesize that in pwMS with high subjective trait fatigue, sensorimotor and sensory gating will be reduced and that task-induced fatigability gating will further disrupt gating.

## 3.5.3 Methods

### 3.5.3.1 Study sample

We initially recruited 38 participants (18 pwMS, 20 HC) in the final analyses of this study (see Table 1 for group characteristics). Inclusion criteria for HC were no history of neurological or psychiatric disorders, no current depression (Beck Depression Inventory II - Fast Screen, BDI-FS $\leq$ 4), and no sleep disorder (Epworth Sleepiness Scale, ESS $\leq$ 10). PwMS were recruited from the outpatient pool of the University Hospital of Magdeburg. They had to be diagnosed with clinically definite MS according to the McDonald criteria and were included when there was a minimum of three months since the last relapse or use of corticosteroids, no current neurological or psychiatric comorbidities as well as no treatment with fatigue or antidepressant medication. Disease-modifying therapy (DMT) consisted of Glatirameracetat ($n$ = 3), Fampridin ($n$ = 3), Siponimod ($n$ = 1), Fingolimod ($n$ = 3), Dimethylfumarat ($n$ = 1), Ocrelizumab ($n$ = 3), and Cladribin ($n$ = 1). Three participants received no DMT. The local ethic committee of the University of Magdeburg approved the study. All participants provided written consent according to the Declaration of Helsinki and received a monetary reward (Euro 30 in total).

**Table 8.** *Baseline group characteristics, mean (± SD).*

| | **pwMS** | **HC** | |
|---|---|---|---|
| gender f/m | 12 / 6 | 13 / 7 | |
| age [years] | 44.61 (12.70) | 47.90 (13.07) | $p = .437$ |
| BDI-FS [points] | 1.33 (1.24) | 1.15 (1.23) | $p = .649$ |
| ESS [points] | 9.17 (4.00) | 5.35 (3.01) | $p = .002$ |
| SDMT [points] | 57.11 (8.62) | 61.05 (6.19) | $p = .112$ |
| $WEIMuS_{total}$ [points] | 29.56 (13.02) | - | |
| $WEIMuS_{cognitive}$ [points] | 15.17 (7.05) | - | |
| disease duration [years] | 14.22 (10.87) | - | |
| EDSS [points] | 3.28 (1.82) | - | |

*BDI-FS,* Becks Depression Inventory – Fast Screen; *EDSS,* Expanded Disability Status Scale; *ESS,* Epworth Sleepiness Scale; *HC,* healthy controls; *pwMS,* people with Multiple Sclerosis; *WEIMuS,* Wuerzburg Fatigue Inventory for Multiple Sclerosis

### 3.5.3.2 Gating paradigms

The PPI paradigm consisted of three conditions: (i) the prepulse-alone condition (80 dB 20 ms white noise bursts, 20 trials), which served as a baseline condition, (ii) the startle-alone condition (105 dB 40 ms white noise bursts, 20 trials), and (iii) the prepulse-startle condition (20 trials) in that the startle stimuli were presented 120 ms after the presentation of the prepulse stimuli. White noise of 70 dB was presented one minute prior to the trials and persisted for the duration of the test as background noise. After the first minute, we presented five startle sounds as habituation stimuli, followed by 60 randomly presented trials, each belonging to one of the above conditions. Both stimuli rise times were near-instantaneous, and the intertrial interval averaged 10 s, ranging from 8 to 12 s.

The standard paired-click paradigm to measure the P50 ERP consisted of 60 pairs of 80 dB white-noise clicks with a duration of 1 ms. The task began with one-minute 30 dB white noise that preceded as background noise. The click pairs were presented with a 500 ms inter-click interval and a random 8-11 s inter-trial interval.

### 3.5.3.3 Procedure

The study began with the participants signing informed consent and completing several questionnaires to assess for demographic information, current mood (BDI-FS) and

daytime sleepiness (ESS). PwMS additionally completed the Wuerzburg Fatigue Inventory (WEIMuS) to assess their subjective trait fatigue. All participants performed the Symbol Digit Modalities Test (SDMT) to assess cognitive functioning. In general, the study consisted of the two gating paradigms (as described above), the fatiguing task, and the subsequent re-presentation of both gating paradigms (see Figure 18). The fatigability-inducing task (an AX- continuous performance task, AX-CPT) consisted of six blocks (B1-B6) á 53 trials (5 minutes). In every trial, four letters were sequentially presented on a black background. A red cue letter, two white distractor letters, and a red probe letter formed one sequence of letters. They were presented for a duration of 300 ms followed by a 1200 ms inter-stimulus interval. The target sequence, when a cue letter "A" was followed by a probe letter "X" (AX-trial), was presented at a 70 % frequency and required pressing the right CTRL-button. Non-target sequences (AY-, BX-, BY- trials) occurred at a 30% frequency and required a left CTRL-button press. For wrong and missed answers, an auditory feedback tone was provided. The blocks were separated by 90 sec breaks. At the beginning, middle, and end of the task, visual analog scales (VAS) were presented. We presented two VAS scales from 0 to 100. One asked the participants "how mentally fit they felt right now at this moment" ($VAS_{fitness}$), and the other asked "how mentally exhausted they felt right now at this moment" ($VAS_{exhaustion}$).

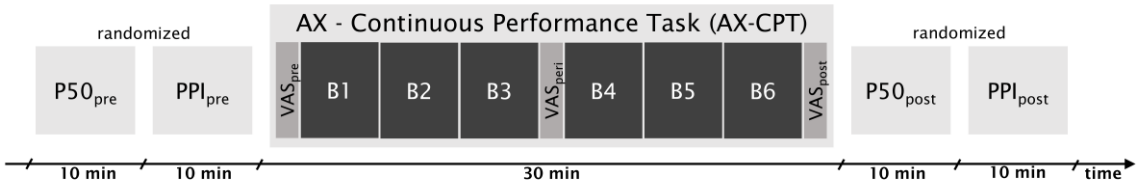


**Figure 18.** Experimental design. After assessing demographic and clinical data via self-report questionnaires, participants performed the auditory P50 sensory gating and prepulse inhibition (PPI) paradigms in a randomized order (pre-session). A 30-minute continuous performance AX-task (AX-CPT task) followed that consisted of six blocks (B1 - B6). Before the first, after the third and at the end, participants were asked about their current perceived fatigue status on visual analog scales ($VAS_{pre}$, $VAS_{peri}$, $VAS_{post}$). Subsequently, the auditory P50 and PPI paradigms were represented in a post-session.

#### 3.5.3.4 EEG signal recording and preprocessing

EEG was recorded at Fz, Cz, and Pz electrodes using Ag/AgCl-electrodes mounted in an elastic cap (EasyCap GmbH, Germany). The ground electrode was attached to the AFz position, and all channels were referenced to the left and right mastoid. An electrooculogram (EOG) of the left eye and an electromyography recording (EMG) of the right eye were recorded. The vertical EOG was placed below the pupil and the horizontal EOG to the external canthus of the left eye. The EMG electrodes were placed over the right orbicularis oculi and a ground electrode on the forehead. The data were recorded by Brain DC amplifier (Brain Products, Germany) and the corresponding software (BrainVision Recorder, version 1.20, Brain Products, Germany) sampled at 1000 Hz. Impedances were kept below 5 kΩ. EEG preprocessing was carried out in BrainVision Analyzer 2.1 (Brain Products, Germany) and was almost identical to the steps described in Linnhoff et al. (2021).

Thus, for the P50 analysis, the EEG data were epoched from -150 to 499 ms post stimulus and then offline band-pass filtered from 1 to 47 Hz. The data was then baseline corrected (-50 to 0 ms), manually inspected for eye-movement artifacts, and averaged. The P50 peak was evaluated at Channel Cz. Peaks were detected as a peak if (i) the P50 peak was the most positive peak occurring 30–80 ms after the stimulus, (ii) the peak was preceded by a negative (Na) and positive (Pa) deflection, and (iii) for the peak detection of the second stimulus (S2) if it occurred within ± 10 ms around the latency of the prior detected peak of the first stimulus (S1). P50 amplitudes were defined as the difference between the P50 peak and the preceding negative trough, separately for S1 and S2. If there was no P50 peak in that range, the P50 amplitude of the second stimuli was scored as 0.01. The P50 suppression was calculated with:

$$( 1 - ( S2 / S1 ) ) \cdot 100$$

Accordingly, higher P50 suppression ratios indicate higher sensory gating, whereas ratios equal to or smaller than zero indicate a higher S2 peak compared to S1 and, thus, no sensory gating. To prevent outliers from distorting group means, we restricted ratios to - 200 % (Thoma et al., 2020). Six participants (5 HC, 1 pwMS) had to be excluded from the P50 gating analysis for not showing sensory gating at the pre-session.

For the PPI analysis, the EMG data were band-pass filtered from 28 to 400 Hz with an additional notch filter of 50 Hz. For each subject, startle responses were segmented for each trial type (-100 to 200 ms after stimulus onset) and then baseline corrected (-100 to 0 ms). Subsequently, the EMG signal was rectified and smoothed with a moving average at a time constant of 11. A manual visual inspection followed, in which all trials featuring excessive noise or a spontaneous blink in the period immediately preceding the stimulus onset were excluded from further analysis. For each trial, the startle response was considered as the maximum blink amplitude in a response window from 20 to 120 ms after stimulus onset. As Van der Linden et al. (2006), we defined a valid startle response as a peak of at least 3 SD above baseline activity. Baseline activity was calculated as the average response to the prepulse in the prepulse-alone trials, except for those trials in which the startling activity caused by the prepulse exceeded 10 µV. The participants had to exhibit at least five startle responses. Otherwise, they were classified as non-responders. This led to the exclusion of five participants (2 HC, 3 pwMS) from the PPI analysis. PPI ratio was calculated with:

$$( ( M_{\text{startle-alone}} - M_{\text{prepulse-startle}} ) / M_{\text{startle-alone}} ) \cdot 100$$

The average includes values of zero for non-responses. Thus, we report PPI magnitudes. Higher PPI ratios indicate higher sensorimotor gating.

#### 3.5.3.5 Statistical analysis

All data analyses were carried out in R Statistical Software (version 4.2.0, R Core Team, 2022). We analyzed the data with Linear Mixed Models (LMMs) using the *lmer* function from the *afex* package (Singmann et al., 2022). P values were obtained using Sattersthwaite´s approximation method. Results are partly described using the *report* package (Makowski et al., 2020). Invalid and error trials were excluded from the reaction time data analysis. Furthermore, to reduce the impact of outliers, we winsorized outliers below or above 1.5 times the interquartile range to this limit. As dependent variables, we used the VAS scores, reaction time variability, as well as PPI and P50 sensory gating ratios. Time, group, and group x time were considered as fixed factors. Data from HC during pre-session or block B1 were used as baseline. Individuals and their variation of the dependent variable were used as random effects. Finally, Pearson correlational

analyses were used to examine the relationship between the subjective trait and state fatigue scores and PPI as well as P50 sensory gating ratios.

## 3.5.4 Results

### 3.5.4.1 Manipulation check

The VAS ratings as a function of time on task separate for HC and pwMS are shown in Figure 19. The LMM to analyze subjective mental fitness ratings ($VAS_{fitness}$) with time on task had a substantial total explanatory power ($R^2_{conditional}$ = 0.886). As hypothesized, the AX-Task led to a significant decrease in $VAS_{fitness}$ ratings with time on task in all participants [$F(1,36) = 18.724$, $p < .001$, $\eta_p^2 = .34$]. PwMS trended to rate their mental fitness lower compared to HC [$F(1,36) = 3.815$, $p = .059$, $\eta_p^2 = .10$]. Importantly, the interaction between *time* and *group* was significant [$F(1,36) = 4.279$, $p = .046$, $\eta_p^2 = .11$]. Thus, the initial $VAS_{fitness}$ ratings for the HC group were 75.51 points [$\beta_{intercept}$, 95% CI (67.05 , 83.97)] and for the pwMS 63.24 points [$\beta_{intercept} + \beta_{group}$ , 95% CI (42.48 , 83.95)]. With each new query, $VAS_{fitness}$ ratings decreased by 3.53 points [$\beta_{time}$, 95% CI (-7.74 , 0.68)] in HC, whereas they decreased by 10.00 points [$\beta_{time} + \beta_{time*group}$ , 95% CI (-20.33 , 0.33)] in pwMS (see Figure 19A).

The LMM to analyze the subjective mental exhaustion ratings ($VAS_{exhaustion}$) revealed a significant overall increase in the $VAS_{exhaustion}$ ratings with time on task [$F(1,36) = 8.608$, $p = .006$, $\eta_p^2 = .19$] and a significant difference between both groups [$F(1,36) = 6.860$, $p = .013$, $\eta_p^2 = .16$]. However, the interaction between *time* and *group* was not significant [$F(1,36) = 0.894$, $p = .351$]. The model explained about 80 % of the variance in mental fitness ratings ($R^2_{conditional}$ = 0.804). The HC group rated their initial mental exhaustion with 24.08 points [$\beta_{intercept}$, 95% CI (14.37 , 33.80)] and the pwMS group with 42.97 points [$\beta_{intercept} + \beta_{group}$ , 95% CI (19.14 , 66.80)]. With each new query, $VAS_{exhaustion}$ ratings increased by 7.10 points [$\beta_{time}$, 95% CI (2.17 , 12.03)] in HC and by 3.64 points in pwMS [$\beta_{time} + \beta_{time*group}$ , 95% CI (-8.45 , 15.73)] in pwMS (see Figure 19B). The following correlational analyses were conducted only with $VAS_{fitness}$ ratings due to the lack of interaction in $VAS_{exhaustion}$ ratings.

Furthermore, we analyzed reaction time variability as an additional behavioral parameter. PwMS tended to have a higher reaction time variability compared to HC [main effect *group*: $F(1,36) = 3.619$, $p = .065$, $\eta_p^2 = .09$]. However, the data showed no significant changes with time on task [*time*: $F(1,36) = 1.495$, $p = .229$; *time* x *group*: $F(1,36) = 1.134$, $p = .294$].

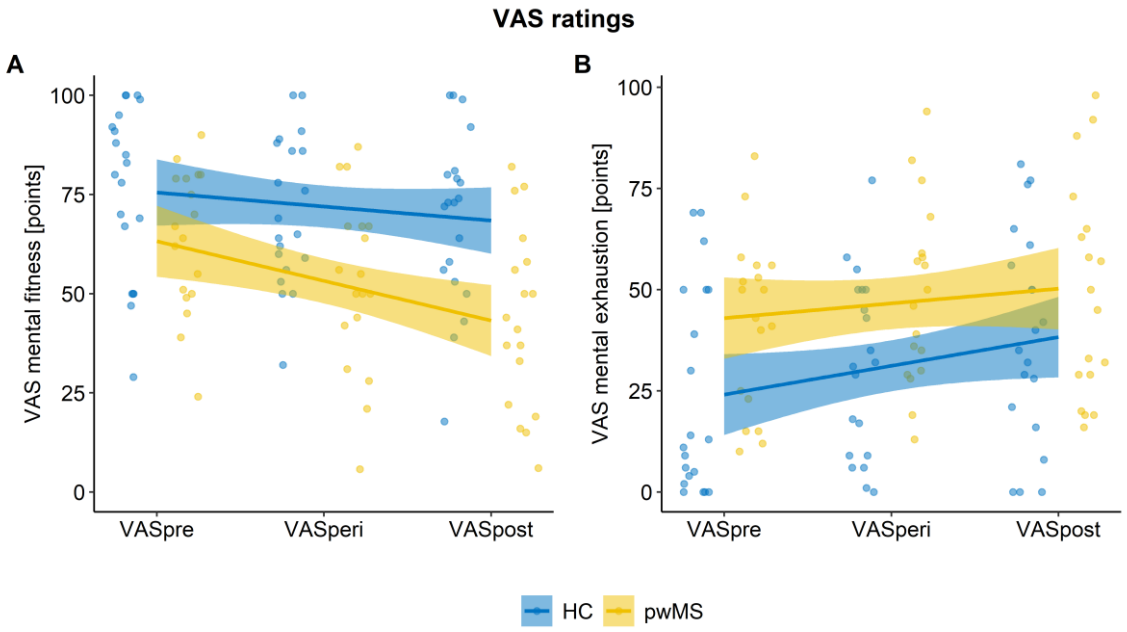


**Figure 19.** Regression plots for the visual analog scale (VAS) ratings of mental fitness (A) and mental exhaustion (B) against the VAS queries ($VAS_{pre}$, $VAS_{peri}$, $VAS_{post}$) separate for the HC and pwMS groups.

### 3.5.4.2 Prepulse inhibition

The LMM to analyze time on task effects on PPI ratios revealed a significant main effect of *time* [$F(1,31) = 7.134$, $p = .012$, $\eta_p^2 = .19$]. Thus, PPI ratios decreased after fatigability induction. However, PPI ratios did not differ between both groups [$F(1,31) = 0.335$, $p = .567$] and likewise the decrease in PPI ratios was similar in both groups [*time* x *group*: $F(1,31) = 0.215$, $p = .646$]. The model had a substantial total explanatory power ($R^2_{conditional} = 0.79$). During the pre-session HC had initial PPI ratios of 73.82 % [$\beta_{intercept}$, 95% CI (63.72 , 83.92)] and pwMS of 77.51 [$\beta_{intercept} + \beta_{group}$ , 95% CI (52.42 , 102.59)].

With time on task, PPI ratios in HC decreased by 5.56 % [$\beta_{time}$, 95% CI (-12.20 , 1.09)] and similarly decreased by 7.90 % [$\beta_{time} + \beta_{time*group}$ , 95% CI (-24.39 , 8.60)] in pwMS (see Figure 20A). Correlational analyses on the relationship between initial PPI ratios and $WEIMuS_{cog}$ scores (subjective trait fatigue scores) as well as between the change in PPI ratios and $VAS_{fitness}$ ratings (subjective state fatigue scores) with time on task revealed no significant associations (all *ps* > .119).

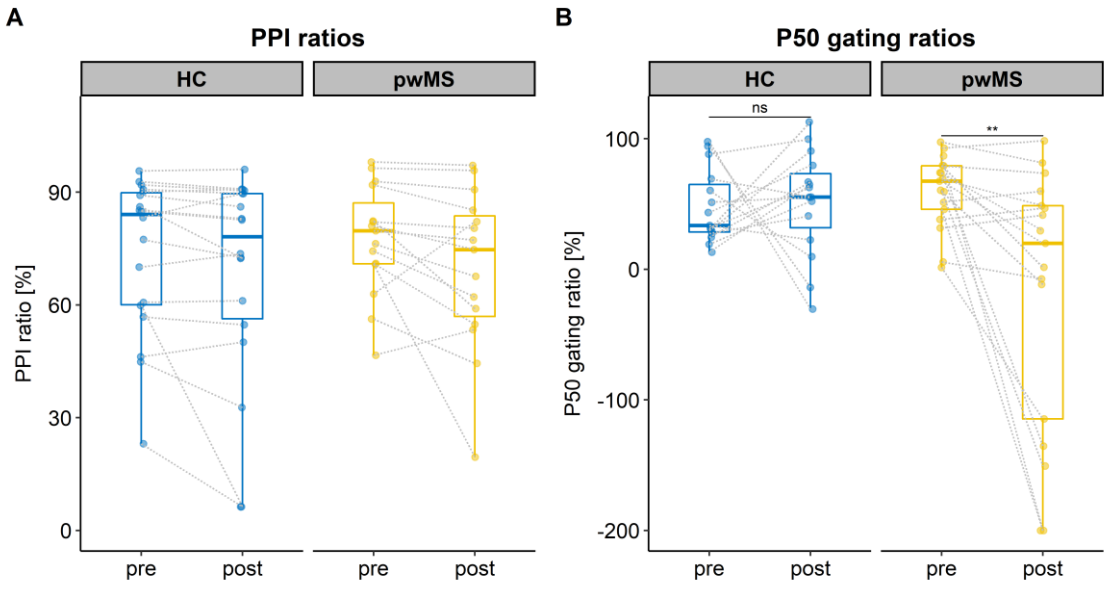


**Figure 20.** PPI (A) and P50 gating ratios (B) as a function of session (pre, post) separate for the HC and pwMS groups.

### 3.5.4.3 P50 sensory gating

The LMM to analyze time on task effects on P50 sensory gating ratios revealed a significant main effect *time* [$F(1,30) = 6.470$, $p = .016$, $\eta_p^2 = .18$] but no significant main effect *group* [$F(1,30) = 1.481$, $p = .233$]. Importantly, we found a significant interaction between *time* and *group* [$F(1,30) = 7.134$, $p = .012$, $\eta_p^2 = .19$]. The model had a moderate explanatory power. It explained about 25 % of the variance in P50 ratios ($R^2_{conditional} = 0.236$). Post-hoc tests revealed a significant decrease of P50 gating ratios in pwMS [$t(16) = 3.264$, $p = .005$, Cohen´s $d = .792$] but not in HC [$t(14) = -0.236$, $p = .817$].

Thus, in HC, P50 ratios slightly increased by 3.63 % [$\beta_{time}$, 95% CI (-37.73 , 44.99)] after fatigability induction, whereas they substantially decreased by 78.35 % [$\beta_{time} + \beta_{time*group}$ , 95% CI (-176.45 , 19.75)] in pwMS (see Figure 20B). Importantly, this decrease was driven by an increase of the S2 amplitude [$t(16) = -3.620$, $p = .002$, Cohen´s $d = -.878$] and not a decrease of S1 amplitude [$t(16) = 1.351$, $p = .195$]. Thus, the time on task effect on gating ratios is not a result of habituation but rather a result of disrupted sensory gating.

Furthermore, we explored the relationship between initial P50 sensory gating ratios and $WEIMuS_{cog}$ scores (subjective trait fatigue scores). The analysis revealed a negative relationship that was, however, not significant [$r(15) = -.382$, $p = .130$]. The scatter plot visually revealed one outlier (see Figure 21A). After removing this outlier, the Pearson's correlation reached significance [$r(15) = -.705$, $p = .002$] suggesting that pwMS with higher $WEIMuS_{cog}$ scores have lower P50 sensory gating ratios at pre-session (see Figure 21A). Finally, we analyzed the relationship between the change in P50 sensory gating ratios with time on task and the change in $VAS_{fitness}$ ratings (subjective state fatigue scores) with time on task. The analysis revealed a significant positive relationship [$r(30) = .375$, $p = .034$]. Thus, the results suggest that participants with a stronger decrease in $VAS_{fitness}$ ratings with time on task tend to have a stronger decrease in P50 sensory gating ratios. Interestingly, this relationship was strongly driven by the HC group (see Figure 21B).

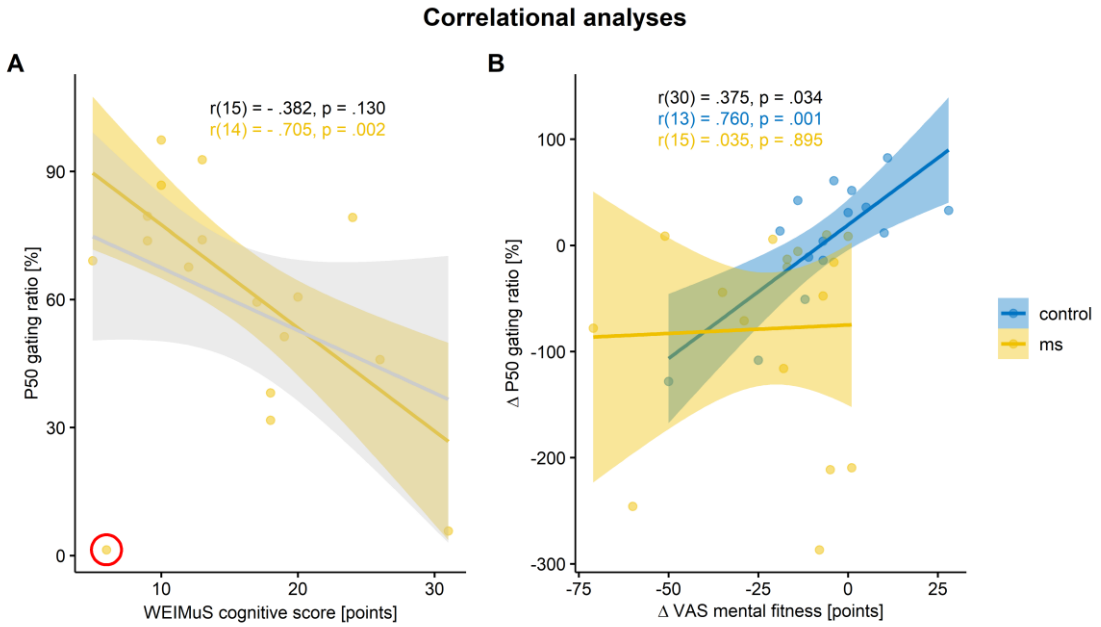


**Figure 21.** Pearson´s correlation analyses to analyze the associations between P50 sensory gating and subjective mental fatigue ratings. A: Association between the P50 gating ratios and WEIMuS cognitive scores at pre-session before (grey) and after (yellow) outlier removal (red circle). B: Association between the P50 gating ratios and WEIMuS cognitive scores at pre-session after outlier removal. C: Association between the change in P50 sensory gating ratios and the change in subjective mental fitness ratings with time on task separate for the HC and pwMS group.

## 3.5.5 Discussion

This study explored fatigue- and fatigability-related changes in PPI and P50 sensory gating ratios in pwMS and HC. We found a significant relationship between P50 sensory gating ratios and subjective trait fatigue scores in pwMS. Furthermore, the AX-CPT task induced a greater decrease of mental fitness in pwMS. While PPI ratios slightly decreased after fatigability induction in both groups, P50 sensory gating ratios strongly decreased only in pwMS. Interestingly, in HC, the change in P50 gating ratios was associated with the change in subjective state fatigue ratings.

#### 3.5.5.1 Fatigability induction

We used two VAS scales to assess the subject´s perceived fatigue status with time on task as well as reaction time variability as behavioral fatigability marker. As hypothesized, pwMS reported feeling less mentally fit with time on task compared to HC. Contrary, ratings on mental exhaustion were higher in pwMS but similarly increased in both groups. Reaction time variability remained stable with time on task. In conclusion, considering that both subjective VAS scales significantly changed with time on task and the known high fatigue scores in pwMS, we assume that the task successfully induced fatigability.

Nevertheless, our results highlight the importance of unified VAS scales to assess subjective state fatigue in pwMS. We presented two VAS scales, one positively and one rather negatively phrased. This increased task engagement but may have also led to socially desirable answers. Additionally, it might have increased self-awareness. Thus, as part of a clinical examination, pwMS are often asked about their current level of exhaustion, which increases their awareness of the syndrome. Consequently, when asked "how exhausted they felt", VAS ratings might have been biased in pwMS. Future studies should consider this and pay attention to uniform VAS scales.

#### 3.5.5.2 Gating deficits

Contrary to our assumption, pwMS and HC had comparable gating ratios during the pre-session. However, results revealed a significant negative correlation between P50 gating ratios and cognitive trait fatigue scores in pwMS. Thus, pwMS with higher trait fatigue showed reduced sensory gating at pre-session. Importantly, we found no associations between clinical data and sensory gating. Thus, the relationship between sensory gating and trait fatigue is not mediated by the EDSS score or the disease duration.

To our knowledge, this is the first study reporting sensory gating deficits in higher fatigued pwMS. Only a few studies investigated electrophysiological components and their association with trait fatigue in pwMS with mixed results. Thus, two studies reported that pwMS had delayed latency in the P300 component of the auditory event-related potential (Chinnadurai et al., 2016; Pokryszko-Dragan et al., 2016). In contrast, Lazarevic et al. (2021) reported no effects of MS-related fatigue on the P300 component. Our findings support the use of sensory gating as a biomarker for trait fatigue to extend

the current subjective diagnosis. Additionally, P50 sensory gating has already been repeatedly presented as a valid and reliable diagnostic parameter for other attention-related deficits (Holstein et al., 2013; Micoulaud-Franchi et al., 2019; Shen et al., 2020; Xia et al., 2020). Therefore, P50 gating deficits are not exclusive to a particular disorder. Instead, they might instead be a general characteristic of attention-related disorders (Holstein et al., 2013). However, further studies are needed to replicate our findings and clarify the role of sensory gating in the assessment of trait fatigue in pwMS. Furthermore, P50 sensory gating strongly decreased after fatigability induction in pwMS and not in HC. Importantly, this effect resulted from larger amplitudes after the second instead of the first click sound. Thus, our finding is not a result of habituation but rather of decreased gating. In some participants, sensory gating even got completely suppressed. Therefore, P50 sensory gating might not only act as a suitable marker for trait fatigue but also for fatigability. Our data are in line with previous studies that also reported gating deficits after fatigability induction (Aleksandrov et al., 2016; Linnhoff et al., 2021). Additionally, we found a positive correlation between the change in P50 suppression and the change in subjective VAS ratings. Interestingly, however, this relationship was primarily found in HC. At the same time, the HC group was unaffected by fatigability. The exact nature of the relationship between objective fatigability and subjective state fatigue remains a still open question. Thus, both might jointly appear or rather exist as two distinct constructs. This might explain why some studies find associations while others do not (see Linnhoff et al., 2019 for a comprehensive review).

Prepulse inhibition, on the other hand, had no relationship with trait fatigue scores. Holstein et al. (2013) similarly reported significant P50 sensory gating deficits in people with schizophrenia while they found no deficits in PPI. The authors argue that P50 suppression and PPI presumably represent different aspects of attention due to their different interstimulus intervals. Consequently, P50 suppression with a longer ISI of 500 ms may have a conceptually more direct relationship with attention than PPI with a shorter ISI of 120 ms. As expected, however, PPI ratios decreased with time on task, as has already been reported in previous studies (Linnhoff et al., 2021; Van der Linden et al., 2006). But pwMS had a similar decrease compared to HC, and we found no association with subjective VAS scores. Similar to the results in our previous study (Linnhoff et al., 2021), we found a relatively small PPI decrease of approximately 6 % in HC and 8 % in pwMS. Contrary, Van der Linden et al. (2006) reported much greater reductions of

approximately 20 %. Thus, the fatigability-related disruption in PPI might be too small to produce group differences.

Nevertheless, the present results highlight the important role of the thalamus in the cortico-striato-thalamo-cortical fatigue network. Different hypotheses have been proposed about the role played by the thalamus in fatigue development. In some studies, activity increases with fatigue, while in others, it decreases (see Capone et al., 2020 for a comprehensive review). Capone et al. (2020) attempted to combine results of the existing literature and postulate that thalamic activity initially increases in a compensatory manner to counteract MS-related structural damage. When plasticity is no longer possible, functional connectivity drops, and fatigue becomes chronic. This initial increased thalamus activity has also been reported in healthy subjects after inducing mental fatigue (Batouli et al., 2020). Taken together, our findings contribute to a better understanding of the pathomechanisms involved in fatigue and fatigability. Consequently, changes in thalamus activity may result in permanent or temporary dysfunction of thalamus-dependent cognitive control mechanisms, such as sensorimotor and sensory gating. It should be noted that the results of our study are correlative, making it impossible to draw conclusions about causality.

#### 3.5.5.3 Limitations

This study has a few limitations. First, the VAS scales were always presented in the same order, which migh have resulted in order effects. However, both VAS scales had different polarization, resulting in an increased task engagement in the participants as they had to read carefully. In addition, we restricted VAS queries to three times in order to reduce face validity. Another limitation is the relatively large number of exclusions in the P50 and PPI gating paradigms, leading to different sample sizes. However, we used this procedure as it is consistent with the common evaluation criteria of PPI and P50 gating ratios, keeping the data comparable. Lastly, due to the lack of structural and functional MRI data, possible correlations with thalamic activity remain purely hypothetical. Future studies should additionally collect thalamic activity and size and investigate associations with trait fatigue and fatigability in pwMS.

#### 3.5.5.4 Conclusion

This is the first study to report fatigue- and fatigability-related sensory gating deficits in pwMS. Especially P50 sensory gating seems to be a suitable marker to complement the subjective fatigue diagnosis. Gating paradigms are independent of learning effects or psychological biases. They are safe to administer and can easily be implemented in the current fatigue diagnostic and therapy monitoring. Additionally, this study gives new insight into the pathomechanisms of fatigability in pwMS and highlights the important role of the thalamus in the fatigue circuit.

# 4 General Discussion

## 4.1 Summary

This thesis aimed to complement the purely subjective fatigue diagnosis with objectively measurable fatigue and fatigability parameters as well as to examine the potential of frontal tDCS for the treatment of fatigability in pwMS. Fatigue is one of the most common symptoms of MS, affecting up to 80% of pwMS (Oliva Ramirez et al., 2021). It drastically reduces the quality of life of pwMS and is the leading cause of early retirement (Oliva Ramirez et al., 2021; Simmons et al., 2010; Yamout et al., 2013). And yet, despite its high clinical and social relevance, progress in understanding and treating MS-related fatigue and fatigability is still sparse.

In this thesis, I first presented a review article in which I developed a unified fatigue taxonomy and emphasized the necessity of a common definition and terminology in order to facilitate future research on the invisible fatigue syndrome. Moreover, in the review article, I presented a comprehensive overview of the objective parameters used to measure fatigability. A wide range of behavioral parameters, including reaction time and accuracy, have been studied. Nevertheless, they are susceptible to learning effects or even ceiling effects when it comes to accuracy, resulting in controversial results. Electrophysiological parameters showed positive results but have not yet been investigated enough to provide reliable results. However, most studies have failed to demonstrate a correlation between subjective fatigue and fatigability, as I have discussed extensively in the review article. Finally, I presented several studies examining the effects of tES on fatigue in pwMS and fatigability in healthy individuals and discussed the potential applications of tES for fatigue and fatigability treatment.

In Project B, I investigated the effects of repetitive tDCS on cognitive fatigue and fatigability in pwMS. During this empirical study, pwMS received either anodal or

sham stimulation twice a week over the DLPFC for four weeks. Both the verum and sham groups showed a reduction in subjective fatigue symptoms. However, the repetitive stimulations did not affect fatigability symptoms.

Project C consisted of three empirical studies exploring reaction time variability and four electrophysiological fatigability candidate markers. In Study C1, I investigated fatigability-related changes in frontomedial theta and occipital alpha power, as well as PPI and sensory gating ratios in healthy subjects while or after they had been fatigued for 90 minutes. Additionally, half of the subjects were anodally stimulated for 30 minutes during the fatiguing task. The results of this study showed that all four parameters changed while or after fatigability induction. Thus, frontomedial theta and occipital alpha power increased with time on task, and both gating indices decreased. I also demonstrated that tDCS counteracted fatigability development, resulting in smaller changes in the fatigability parameters compared with the sham group. As a final result, I found a correlative relationship between subjective state fatigue and theta power, in which subjects who were more fatigued showed a greater increase in theta power.

In study C2, I examined frontomedial theta and occipital alpha power in fatigued pwMS and age-matched controls. The results showed no initial differences in oscillatory brain activity between both groups. However, pwMS showed greater fatigability during the task, as evidenced by both subjective VAS scores and increased reaction time variability. Interestingly, results varied depending on how the VAS scales were phrased. Frontomedial theta power did not increase significantly over time. Alpha power, on the other hand, increased significantly in pwMS while remaining stable in HC. Finally, I found no correlation between subjective state and trait fatigue and fatigability-related changes.

Finally, in Study C3, I investigated PPI and sensory gating ratios in pwMS and age-matched controls. Gating indices were assessed before and after a 30-minute exhaustive task. PwMS demonstrated greater fatigability induction as indicated by subjective VAS scores. However, as in Study C2, the phrasing of the VAS items was crucial. Reaction time variability did not increase significantly in this study. Both groups had similar initial gating ratios. However, interestingly, sensory gating ratios and WEIMuS scores were negatively correlated in pwMS, indicating lower initial gating in

pwMS with higher trait fatigue scores. After fatigability induction, both gating parameters decreased significantly. However, only the P50 gating ratio also showed a significant interaction effect that resulted from a significant decline of ratios in pwMS alone. Interestingly, the change in P50 gating was associated with the difference in subjective VAS scores but only in healthy controls.

In summary, in this thesis, I have demonstrated the importance of distinguishing subjective trait fatigue from state fatigue as well as fatigability in pwMS. By consistently paying attention to the terminology, I presented four electrophysiological markers that are sensitive to fatigability and partly related to subjective fatigue. Moreover, I demonstrated the effect of differently phrased VAS items on the results. Finally, I showed that a single session of anodal tDCS counteracted fatigability induction in healthy subjects, whereas repetitive tDCS did not affect fatigability in pwMS. The results will be discussed in more detail and integrated into the existing literature in the following section.

## 4.2 Objective assessment of fatigue and fatigability

In this thesis, I presented several behavioral and electrophysiological candidate markers for measuring fatigue and fatigability in pwMS and healthy controls.

In two empirical studies (Study B1 and Study C2), reaction time variability increased with time on task. Subjectively more fatigued pwMS showed a significantly greater increase in reaction time variability compared to HC. However, no empirical study in this thesis found a relationship between reaction time variability and subjective trait or state fatigue. It has been suggested that reaction time variability may be a finer-grained behavioral marker and more sensitive to the small fatigability-related changes. Thus, Bruce et al. (2010) described that fatigue may result in occasional lapses in attention, which might be more reliably detected in the variation of reaction times instead of a linear increase. Indeed, reaction time variability has been shown to be a reliable marker of attention disorders such as ADHD (Tamm et al., 2012). However, it has rarely been studied in MS-related fatigue and with controversial results. Thus, while Bodling et al. (2012) found an increase with increasing cognitive load, Bruce et al. (2010) did not. Therefore, it remains to be determined whether reaction time variability can be used as an objective indicator of fatigability in pwMS.

In Study B1, I examined the P300 amplitude. However, contrary to my hypothesis, I could not demonstrate fatigability-related variations in the P300 amplitude. Thus, P300 amplitudes did not increase with time on task, nor were they influenced by the repetitive tDCS sessions. I investigated the P300 EKP component because it is considered to represent cognitive resources available to update expectancy calculations (Polich, 2007). Previous studies have already demonstrated smaller P300 peak amplitudes associated with subjective trait fatigue in pwMS (Chinnadurai et al., 2016; Fiene et al., 2018; Pokryszko-Dragan et al., 2016). Furthermore, Fiene et al. (2018) demonstrated that a single session of tDCS counteracted the decline in P300 amplitudes. In my study, however, repetitive tDCS sessions did not affect P300 amplitudes. This may be a result of the study design I used. Thus, I investigated the changes in P300 amplitudes over a prolonged period of time rather than during a sustained mental effort. But, as I emphasized in my review, fatigability is better induced during sustained attention tasks since they depend on a high level of endogenous attention (Linnhoff et al., 2019). Therefore, the lack of fatigability induction in Study B may result from a less optimal study design. Consequently, during the three subsequent studies of Project C, I assessed fatigability during sustained mental effort and found that the results were more reliable. Nevertheless, Fiene et al. (2018) used the same study design and reported decreasing P300 amplitudes and increasing latencies. Therefore, it remains unclear whether P300 is a suitable candidate marker. This needs to be investigated in further studies.

Project C focused on the investigation of oscillatory brain activity changes and gating deficits as a result of fatigue and fatigability in healthy subjects and pwMS. In line with my hypotheses, I consistently showed increased low-frequency power and gating deficits after fatigability induction.

Oscillatory brain wave changes have been repeatedly demonstrated in the context of mental fatigue in healthy subjects (Boksem et al., 2005; Craig et al., 2012; Wascher et al., 2014). Thus, frontomedial theta power and occipital alpha power increase with time on task. According to Clayton et al. (2015), sustained attention leads to an increasing mismatch between available and required cognitive resources, which is reflected in the increase in frontomedial theta activity. At the same time, there is an inhibitory alpha increase over task-relevant areas, ergo, in occipital areas during a visual task. However, the causal relationships behind these pathomechanisms have not yet been

clarified. The results of Study C1 and C2 replicated these previous findings. Importantly, I demonstrated that a single session of anodal tDCS in healthy subjects counteracted the occipital alpha increase. Study C2 further demonstrated that the initial resting brain activity in pwMS is similar to healthy subjects and not associated with subjective trait fatigue. However, pwMS demonstrated a stronger alpha power increase with time on task. Frontomedial theta power, on the other hand, remained unchanged in pwMS. Referring to Clayton´s model (Clayton et al., 2015), this might suggest that pwMS have limited top-down control processes, which may lead to abnormal brain activity. This hypothesis is further supported by neuroimaging results, demonstrating that pwMS, contrary to HC, recruit more posterior brain regions for high cognitive load conditions with no improvement in speed compared to healthy controls that recruit more anterior areas to meet task demands (Chen et al., 2020). The results of studies C1 and C2 provide important insights into the pathological processes during the development of fatigability. Frontomedial theta and occipital alpha power may not be suitable as sole markers for an objective fatigue diagnosis. However, they may serve as valuable complements to other objective markers.

Study C1 and Study C3 focused on the effects of fatigue and fatigability on PPI and P50 sensory gating ratios. In both empirical studies, gating was reduced after fatigability induction in healthy controls and pwMS. Especially P50 sensory gating showed a significant difference between pwMS and healthy controls. Previous studies already reported fatigability-related gating deficits in healthy controls (Aleksandrov et al., 2016; Linnhoff et al., 2021; van der Linden et al., 2006). However, I first presented evidence that P50 sensory gating is associated with subjective trait fatigue and is reduced in pwMS after fatigability induction. The results support a thalamic relevance in the fatigue network, as has been discussed in previous studies (Capone et al., 2020). According to the results of my studies, P50 sensory gating might act as a suitable candidate marker for fatigue and fatigability in pwMS. It has already been used as a diagnostic marker for other attention-related disorders (Holstein et al., 2013; Micoulaud-Franchi et al., 2019; Shen et al., 2020; Xia et al., 2020). As sensory gating is easy to assess and evaluate, it could be easily incorporated into a clinical examination.

In summary, I have presented several objective parameters in this thesis. Some of these measures have been more successful than others in measuring fatigability. This

thesis provides only a first step in complementing fatigue diagnosis. However, particularly in Project C, I was able to present valuable and reliable candidate markers for the fatigability diagnosis, both in healthy individuals and in pwMS. It remains unclear, however, to what extent the parameters are useful in clinical settings because they do not always correlate with subjective fatigue. This will be discussed in the next chapter.

## 4.3 Relationship between subjective fatigue and fatigability

In this thesis, the objective parameters investigated were rarely associated with subjective fatigue feelings. In healthy subjects, I found a significant correlation between increased theta power and subjective state fatigue. Additionally, I found a correlation between the decrease in P50 gating ratios and the decrease in subjective mental fitness. In pwMS, I found a significant correlation between baseline gating ratios and subjective trait fatigue.

The results are consistent with numerous other studies that find no relationship between subjective fatigue and fatigability, which I have extensively discussed in my review article (Linnhoff et al., 2019). In fatigue research, one of the major obstacles is that fatigue and fatigability are poorly defined psychological constructs that are difficult to differentiate from other psychological constructs. This makes fatigue measurement very challenging. Consequently, as part of this thesis, I have presented a unified taxonomy and emphasized the need to apply it consistently.

Moreover, there are many problems with the questionnaires used to assess subjective trait fatigue, including poor correlations between the questionnaires and their susceptibility to psychological biases. Furthermore, there are over 250 scales to measure fatigue (Hjollund et al., 2007), resulting in a wide range of questionnaires used in fatigue research. Some of these scales are highly correlated with depression or sleep disorders, resulting in a low degree of specificity (Hjollund et al., 2007). On the other hand, the possibilities for assessing subjective state fatigue are limited. Numerical ratings or visual analog scales have been used. They are particularly useful for directly assessing a current level of subjective exhaustion. However, as of now, there are no standardized VAS scales to measure subjective feelings of fatigue, leaving each study group to develop their own

VAS items. My thesis demonstrated that this can be of decisive importance regarding the results. Thus, I found that asking in a positive manner how "mentally fit" the subjects were "right now at this moment" was significantly different from asking in a negative manner how "mentally exhausted" the subjects were "right now at this moment". At first glance, asking about the subjectively perceived exhaustion may appear more reasonable. However, this may lead to priming effects, especially in pwMS, who are frequently exposed to the terms "exhaustion" or "fatigue" during clinical examinations. Thus, pwMS may already have a much higher interoception and tendency to rate exhaustion higher, which was confirmed in my studies. Consequently, future studies should pay attention to uniform fatigue scales and reduce the number of available scales to a few good ones. Particularly, MFIS is used internationally and has been evaluated with large sample sizes (Strober et al., 2020). In this thesis, I used the WEIMuS (Flachenecker et al., 2006) because it was developed especially for the German-speaking area. However, the MFIS may be more suitable for better comparability in future studies.

In this regard, the fact that we sometimes find correlations and sometimes do not confirms the assumption that fatigue and fatigability appear to be two separate constructs, able to occur independently as well as together. In theory, patients who suffer from subjective trait fatigue should exhibit measurable deficits. But based on current research, this does not seem to be the case. However, it is essential to note that an experiment is always an artificial situation that is also limited in time. Thus, fatigue is associated with motivational aspects (Herlambang et al., 2021), which are usually high during participation in an experiment. It may be that the subjects simply try very hard to do well during the execution of the task, and they manage to do so for the short duration of a study. However, maintaining a high motivational state during everyday life or at work requires a considerable amount of resources and can not be sustained for long.

## 4.4 tDCS treatment

Contrary to my hypothesis, I did not observe a decrease in fatigability symptoms in pwMS following repetitive tDCS sessions, which neither confirms nor contradicts the previous literature. Therefore, some studies have shown that repetitive tDCS can improve subjective trait fatigue (Ayache et al., 2016; Ayache et al., 2017; Cancelli et al., 2018; Chalah, Lefaucheur, & Ayache, 2017; Chalah, Riachi, et al., 2017; Charvet et al., 2018;

Ferrucci et al., 2014; Saiote et al., 2014; Tecchio et al., 2014; Tecchio et al., 2015; Workman et al., 2020), while others have shown that a single tDCS session can improve fatigability in healthy subjects (McIntire et al., 2014; McIntire et al., 2017; Nelson et al., 2014) as well as in pwMS (Fiene et al., 2018). It is important to note that in these studies, stimulation was administered when the patients performed a task rather than offline while resting. This being said, in Study C1, I found a positive effect of a single session of tDCS when young, healthy subjects were performing an exhaustive task. Future studies should revert to the established study design of five consecutive days and, at best, use concurrent cognitive training to improve fatigability, as was done by Charvet et al. (2018).

In order to modify the abnormal brain activity associated with fatigability, tACS may be a more effective approach. Thus, in project C, I demonstrated that theta activity in the frontomedial region increased with increasing subjective state fatigue, as did alpha power in the occipital region. In PwMS, alpha power was also increased, but theta power was not. Theta power plays a central role in monitoring cognitive processes. Thus, the frontomedial application of theta-tACS might enhance cognitive control and counteract the performance decline with time-on-task, as suggested by Clayton et al. (2015). Additionally, gamma-tACS may be used to reduce occipital alpha oscillations via cross-frequency coupling. To date, however, tACS has been used sparingly and only in healthy subjects to treat fatigability. Loeffler et al. (2018) applied 40 Hz gamma-tACS and effectively counteracted the increase in reaction time with time on task. In contrast, two other studies applied alpha-tACS at 10 Hz over the occipitoparietal cortex (Clayton et al., 2018, 2019). The stimulation stabilized visual attention and prevented the deterioration of visual performance. Thus, alpha activity does not inevitably reflect decreased attention which once more illustrates the complexity of fatigue pathomechanisms.

## 4.5 Fatigue and Fatigability in other neurological diseases

Although I have concentrated on fatigue in pwMS in this thesis, fatigue is a syndrome that occurs in a wide variety of neurological disorders. Thus, fatigue has been associated with Parkinson's disease, stroke, traumatic brain injury, amyotrophic lateral sclerosis,

cancer, and many others. Additionally, fatigue can exist independently of an underlying neurological condition, in which case it is referred to as chronic fatigue syndrome.

It is beyond the scope of this thesis to discuss the pathophysiology of all the underlying disorders. There are, however, some overlapping findings. These include increased levels of proinflammatory cytokines and an underlying prefrontal pathology (Kluger et al., 2013; Penner & Paul, 2017; Zaehle, 2021). Nevertheless, there have been very few reviews and no empirical studies that have been conducted with different fatigue groups. Thus, Penner describes the current research status on fatigue as "least-studied" and "least-understood" (Penner & Paul, 2017). It remains an open question whether all of these fatigue syndromes of the different underlying diseases share the same etiology. As fatigue is similarly described in all these disorders, it is reasonable to assume this is the case. However, a lot of future research is required to answer this question.

Currently, fatigue is once again gaining sad notoriety. As with pwMS, fatigue is one of the most commonly reported symptoms of Long Covid (Nalbandian et al., 2021), causing severe limitations to the quality of life for individuals (Dressing et al., 2022; Malik et al., 2022). Consequently, at the end of my thesis, I have written a further review article integrating my knowledge of fatigue in MS and the potential of using non-invasive brain stimulation (NIBS) as an alternative treatment approach into the current state of research regarding Long Covid (Linnhoff et al., under review D). As in MS-related fatigue, the exact pathogenic mechanisms underlying the fatigue development in Long Covid are yet not fully understood. Three influential core hypotheses have been proposed: Long Covid-related fatigue (L-COF) has been related to (i) a dysregulated immune-system after an acute Covid-19 infection (Oronsky et al., 2021; Ramakrishnan et al., 2021), (ii) frontal hypometabolism (Blazhenets et al., 2021; Hosp et al., 2021), and (iii) reduction in cerebral-blood flow (Qin et al., 2021; van Campen et al., 2021). NIBS techniques, such as transcutaneous auricular vagus nerve stimulation (taVNS), tDCS, and tACS, might be used to selectively modulate maladaptive fatigue-related neuronal or immunological activity reported in L-COF. Several Case Reports already exist that present preliminary evidence for the effectiveness of tDCS and taVNS on L-COF (Badran et al., 2022; Eilam-Stock et al., 2021; Gómez et al., 2021). An overview of the NIBS techniques proposed to treat the different pathomechanisms underlying L-COF is illustrated in Figure 22.

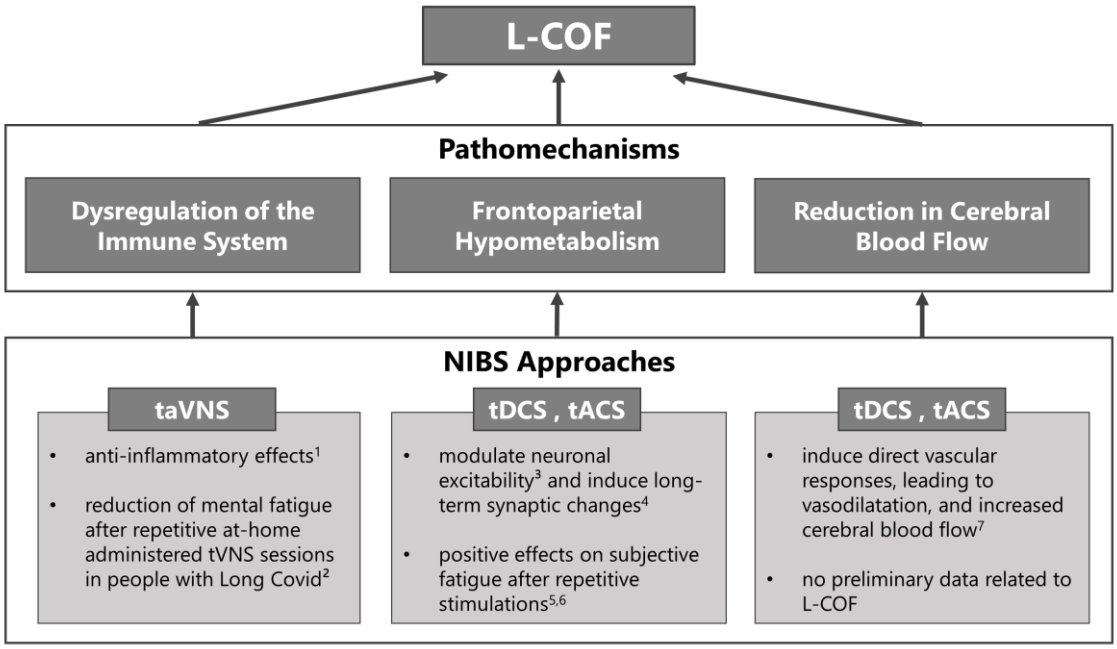


**Figure 22.** Non-invasive brain stimulation (NIBS) approaches for the treatment of individual pathomechanisms of Long-Covid-related fatigue (L-COF). Taken from Linnhoff et al. (under review D).

Transcutaneous auricular vagus nerve stimulation (taVNS) has been shown to have anti-inflammatory effects through its efferent projections, the so-called cholinergic anti-inflammatory pathway ([1] Kelly et al., 2022), and could have a stabilizing effect on the dysregulated immune-system after an acute Covid-19 infection that could lead to L-COF. First positive effects support this hypothesis and show a reduction of mental fatigue in people with L-COF after repetitive taVNS sessions ([2] Badran et al., 2022). Transcranial direct current stimulation (tDCS) and alternating current stimulation (tACS) have been shown to modulate neuronal responsiveness ([3] Reed & Cohen Kadosh, 2018) and to induce long-term effects via long-term potentiation ([4] Monte-Silva et al., 2013). They could therefore be used to counteract the observed frontoparietal hypometabolism after an acute Covid-19 infection that has been associated with L-COF. Preliminary data to support this hypotheses show positive effects on self-reported fatigue scores after repetitive sessions in people with L-COF ([5] Gómez et al., 2021, [6] Eilam-Stock et al., 2021). Following neuronal acitivity or via direct vascular responses, tDCS and tACS have also been shown to increase cerebral blood flow ([7] Bahr-Hosseini & Bikson, 2021). Therefore they might also be an optimal strategy to counteract the observed blood flow reduction in people with L-COF. However, while several data exists that has shown increased cerebral blood flow after tDCS and tACS in healthy subjects and other neurological diseases, no data exists for L-COF.

There is no doubt that fatigue is multifaceted and a syndrome of many neurological diseases. The current literature is heterogeneous and controversial, yet there is significant social and clinical relevance to examine the fatigue causes and possible treatment options. Although many scientists have attempted to study fatigue syndrome in its entirety, their progress has been limited so far. The results and conclusions of this thesis contribute to a better understanding of the pathological changes in fatigue and fatigability in pwMS. However, there are still many questions that remain unanswered.

## 4.6 Limitations

This thesis is not without limitations. First, especially in Project B, it would have been desirable to examine a larger and more homogeneous MS sample. Unfortunately, this was not possible. Especially with clinical subgroups, repetitive testing is often a limiting factor in subject recruitment. For example, some pwMS are physically incapable of attending hospitals on a regular basis. Additionally, pwMS are generally middle-aged and employed, although fatigue symptoms may have limited their employment options. Consequently, scheduling appointments with many participants was challenging, resulting in a smaller sample size. Nevertheless, it should be noted that the sample size is comparable to the majority of former studies on that topic.

Second, the inclusion criteria of pwMS differed between the individual empirical studies. For example, in Studies B1 and C2, I also included pwMS with higher BDI scores, whereas in Study C3, only non-depressed pwMS were included. This was done to increase the study's external validity and ensure that as many patients as possible were included by reducing the inclusion criteria to a minimum. Given the comorbid nature of depression and fatigue, as well as the fact that the questionnaires use some of the same items (see above), it can be very challenging to reduce the inclusion criteria to a cutoff value. Nevertheless, it is important to note that participants in any study were not allowed to have been diagnosed with an acute depressive episode or to take antidepressants.

Finally, I included only pwMS with subjective trait fatigue. Due to organizational and time-dependent factors, I was unable to include both pwMS without fatigue and pwMS with known fatigability, although this would have been desirable. There are, however, limited options to measure fatigability subjectively. One

questionnaire available is the Pittsburgh Fatigability Scale (Glynn et al., 2015; Renner et al., 2021). It measures perceived fatigability in everyday activities and, therefore, might have been more appropriate. However, it has not yet been used in pwMS.

## 4.7 Conclusions

Taken together, in this thesis I have emphasized the importance of a unified fatigue taxonomy as well as the distinction between fatigue and fatigability. To complement the subjective fatigue diagnosis, I presented objective, electrophysiological parameters that can be used to assess fatigue and to evaluate the efficacy of tDCS as an alternative non-pharmacological treatment approach for fatigue and fatigability. Consequently, this thesis contributes to our understanding of the fatigue syndrome in pwMS as well as other neurological diseases. Considering the high social relevance of the research topic as well as the current threat of Long Covid-related fatigue, the results of my thesis provide an important foundation for future research on pathomechanisms and treatment approaches of fatigue and fatigability.

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